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ERRATA

Page 55, line 2 should read "cause they caused severe injury to plants: triethylene glycol dichloride (pure),"

Page 191, under METHODS, line 3, "tests" should read "test"

Page 200, entry 4 in LITERATURE CITED, "IT" in left margin should be deleted and placed in at entry 5, "Hitchcock, A. E., and P. W. Zimmerman."

Page 259, line 18, "*fumentocea*" should read "*frumentacea*"

Page 287, running title, "Seettrstrom" should read "Setterstrom"

Page 365, line 19, "meta" should read "methyl"

ALSO NOTE ERRATA TO VOLUME 9 AS FOLLOWS

Page 197, entry 6 in LITERATURE CITED, "Paris" should read "Praxis"

Page 249, line 13 from bottom, "40 mm." should read "400 mm."

Page 515, entry 45 in LITERATURE CITED, "acidity" should read "activity" (*Note:* This appeared in the American Journal of Botany abstracts as "acidity" and hence had to be so cited.)

MODIFIED STORAGE ORGANS IN HELIANTHUS TUBEROSUS

P. W. ZIMMERMAN AND A. E. HITCHCOCK

Under normal growing conditions *Helianthus tuberosus* L. grows rhizomes during long summer days and then forms storage organs (tubers) on the end of these underground stems as the days become short in the autumn.

It has been demonstrated that *Helianthus* can be induced to tuberize at any time of the year by subjecting the plant to short photoperiods instead of the normal long days of summer. Similar results were induced by capping the stem tips with black cloth from 5:00 p.m. to 9:00 a.m. (2), indicating that the growing tip, in some way, exerts a regulatory influence over tuberization of underground organs.

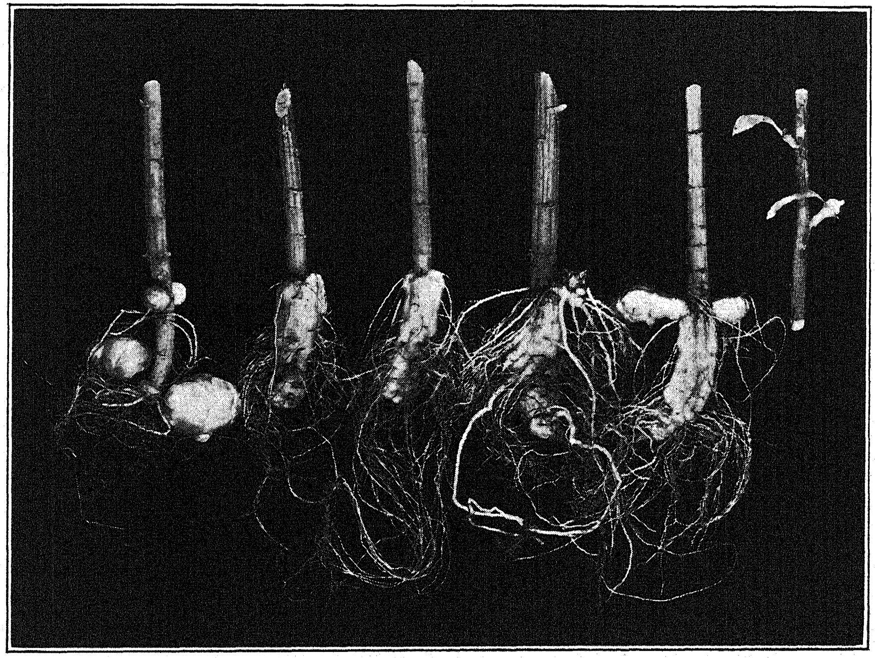

FIGURE 1. Modified storage organs of *Helianthus tuberosus* from cuttings made June 10 and grown in pots until November 9 when the photograph was taken. Left to right: (1) control with all buds attached; (2) and (3) all buds below ground level were removed; (4) and (5) all buds on lower part of stem were removed; buds near ground level remained and formed tubers; (6) buds some distance above ground had a tendency to form tubers.

Rhizomes are underground stems which arise from opposite buds occurring at the base of the stem. As a rule many short nodes, and consequently buds, are formed underground as the shoot grows from an old tuber. The underground buds remain inactive during the early part of the summer but later produce rhizomes from which the tubers arise when days become short enough.

The question arose as to what would become of the manufactured food under short-day conditions if the underground buds were removed to prevent the formation of rhizomes and tubers. Accordingly new plants were made from cuttings of stem tips taken in June. The lowermost buds were removed in some cases so that when the rooted cuttings were planted there were no buds underground. These were compared with cuttings having all buds attached. The plants were grown during the summer in eight-inch clay pots out-of-doors. They grew from three to five feet in height and flowered during October. On November 9 all plants were removed from the pots and the underground parts inspected. The results varied somewhat with the size and condition of the plants. Having been planted late in the season, the controls did not produce the characteristic long rhizomes, tubers being formed near the stem. In general, however, plants with intact underground buds were alike in producing tubers which appeared small but normal. Plants with buds removed stored their food in the basal part of the stem which became fleshy. Where buds remained near the ground level tubers were formed, but the basal part of the stem also became a storage organ. In a few cases buds above the ground grew abnormally large and resembled small storage organs. The results are illustrated in Figure 1.

When the fleshy part of the stem was sectioned it was apparent that the cortex had become storage tissue. In texture it resembled the tubers. The surface of the fleshy stem was more rough than the tuber. The color was white or red, whichever was characteristic of the storage organ of the variety.

The fleshy stems and tubers were stored in a cold frame during the winter. In April they were planted in pots in the greenhouse. The tubers grew as usual. The fleshy stems which had buds remaining a short distance above the storage tissue also produced new plants. No adventitious buds were formed. Where all buds were removed from the stem the fleshy tissue was unable to reproduce the plant. This budless organ would be similar to a tuber with all the buds removed. Like the storage root of dahlia, if no stem tissue with a bud remains, the storage organ is unable to reproduce the plant.

The plants grown from cuttings with all buds attached grew tubers and at harvest time the stems had withered and were dry. In contrast to this, the stems which became fleshy at the base remained green and were apparently in good condition. The buds immediately above the fleshy part were not active but remained in good condition. They resembled buds on dahlia stems closely associated with a storage root (1). In a few cases, however, there was a tendency for buds above the ground to form small tubers.

From the results of these experiments it appears that the regulating force which causes rhizomes to form tubers becomes active at the base of the stem when no rhizomes are present. Whatever the force, the influence was extended only to stem tissue. In other experiments where entire tubers were planted late in the season there was a tendency to form storage roots on the developing parts near the tuber.

SUMMARY

Under normal conditions tubers of *Helianthus tuberosus* are formed in the autumn on rhizomes which arise from underground buds. Plants were grown from cuttings with buds removed from the underground part so they could not give rise to rhizomes. In the absence of rhizomes the basal part of the stem became fleshy and served as a storage organ. When buds above the fleshy part remained attached they grew when the storage tissue was planted the following spring.

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RESPONSE OF GLADIOLUS CORMS TO GROWTH SUBSTANCES

P. W. ZIMMERMAN AND A. E. HITCHCOCK

Our attention was recently called to the fact that corms of gladiolus frequently fail to produce roots or in some cases the few roots produced are not sufficient to support a thrifty plant. Since root-inducing substances were known to be effective on bulbs, tubers, and cuttings of many species, it occurred to us that corms also might respond to chemical treatment. A poor lot of corms which had been fumigated to kill thrips was tested first in the summer of 1937 with varying results. Some interesting responses, however, were apparent and additional tests have recently been made with several varieties of gladiolus. The purpose of this paper is to report the effect of three of the most effective growth substances on root and shoot growth in gladiolus.

MATERIALS AND METHODS

Three named and two unidentified varieties of *Gladiolus* sp. were employed in the experiments. The known varieties were Gold Eagle, Orange Queen, and White Butterfly. White Butterfly and one unidentified variety were supplied while still dormant by Forman T. McLean. The others were purchased from Vaughan's Seed Store.

The growth substances used were naphthaleneacetic acid, indolebutyric acid, and indoleacetic acid. These were used as water solutions in concentrations ranging from 50 mg. to 200 mg. per liter of water. The corms were immersed in the solution for 24 to 72 hours. In some cases the corms were aerated with oxygen gas and carbon dioxide gas while in the solution. One set of Orange Queen and Gold Eagle corms were subjected to a vacuum of 600 mm. for 30 minutes while in the solution.

After the chemical treatment the corms were planted in the greenhouse in a mixture of sand and moss peat so that they could be easily inspected at intervals.

STRUCTURE AND NORMAL DEVELOPMENT

According to Pfeiffer (2) the gladiolus corm consists of the lower swollen internodes of the shoot axis which produces a storage organ capable of reproducing the plant. The buds lie on alternate sides in one vertical plane and bisect the corm. The largest bud is the uppermost which usually produces the largest shoot. If this bud is removed the second bud produces a strong shoot and even the smallest buds may develop.

Generally elongation of the shoot precedes root development. Normally the roots develop in a circle around the basal part of the central axis but not from the other parts of the corm (Fig. 1 B).

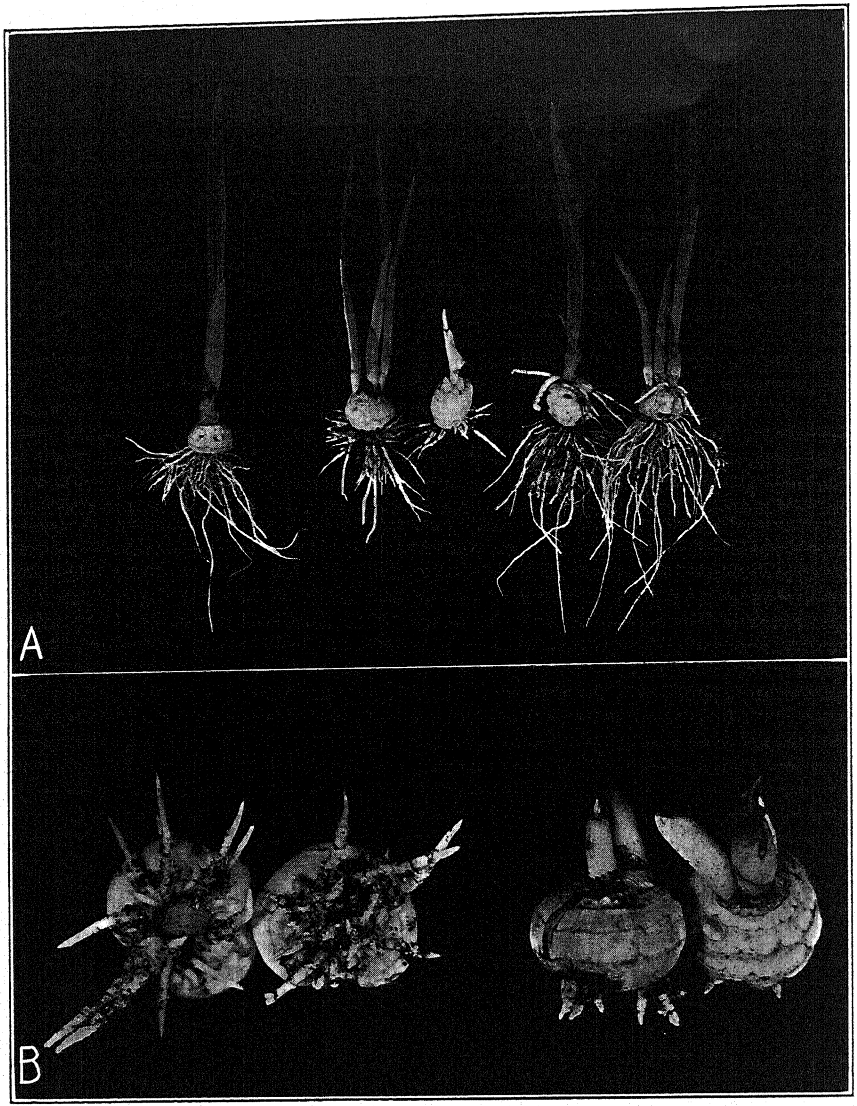

FIGURE 1. Gladiolus (Gold Eagle) showing the effects of growth substances on rooting. The treatments consisted of immersing the corms in the chemical solution for 30 minutes, during which time the vacuum was applied to the extent of 400 to 600 mm. Description reads, left to right: (A) Control; treated with naphthaleneacetic acid, 50 mg. per liter of water; same except 100 mg. per liter; treated with indolebutyric acid, 100 mg. per liter; same except 200 mg. per liter. Note the fasciated contractile roots and retardation of shoot where treated with naphthaleneacetic acid. All photographs taken 14 days after treatment, May 31. (B) Four corms treated with naphthaleneacetic acid, 100 mg. per liter. Left, two corms showing fleshy roots arising around the base of central axis; right, two corms showing sheath leaves developing fleshy instead of scale-like. Retardation of shoot growth is evident.

New corms are formed at the base of the shoot and mature after flowering time. As the corm is developing, contractile (storage) roots grow from the basal part and later serve to anchor the body and to bring the corm to an appropriate level in the soil. Contractile roots are, when young, plump and much larger in diameter than roots of the old corm, showing that they have a different function.

According to Pfeiffer (2) new corms and contractile roots form within 60 days after planting the old corms in the fall. At the same time the floral axis also develops. Unpublished data of Pfeiffer show that when the old corms were planted in the spring contractile roots were initiated in less than 30 days.

EXPERIMENTAL RESULTS

The most striking effects of chemical treatment of gladiolus corms were development of fleshy instead of fibrous roots around the basal axis, the induction of adventitious roots from the corm in line with the leaf scars, the internal development of adventitious roots in large numbers occupying practically the entire corm, the induction of contractile roots even before the shoot develops, and "fasciation" of contractile roots forming a complete ring around the new shoot.

The responses of the corms varied with the concentrations and the substances employed. For example, naphthaleneacetic acid induced fleshy roots at the base of the corm (Fig. 1) while the basal roots of corms treated with the indole derivatives resembled those of the check. Both naphthaleneacetic acid and indolebutyric acid induced contractile roots, though the naphthalene compound was the more effective and had a tendency to cause fasciation. Fifty milligrams of naphthaleneacetic acid per liter of water were fully as effective as 200 mg. per liter of indolebutyric acid. Indoleacetic acid was not as affective for inducing roots as indolebutyric acid.

Figures 1 and 2 show the contractile roots and basal roots induced in 14 days with naphthaleneacetic acid and indolebutyric acid in contrast with controls. These pictures also show fasciated roots and retardation of shoots when 100 mg. per liter of naphthaleneacetic acid were used in the treatment. The number of shoots shown in the treated lots should not be taken as an indication that an abnormally large number of buds had been forced. Some of the controls showed three shoots also. To illustrate the results described, the best specimens were selected from the lot for the pictures.

Applying a 600 mm. vacuum for 30 minutes while the corms were immersed in the solution had approximately the same effect as simply immersing for 48 hours. Possibly lower concentrations would be very effective if the vacuum were applied for several hours.

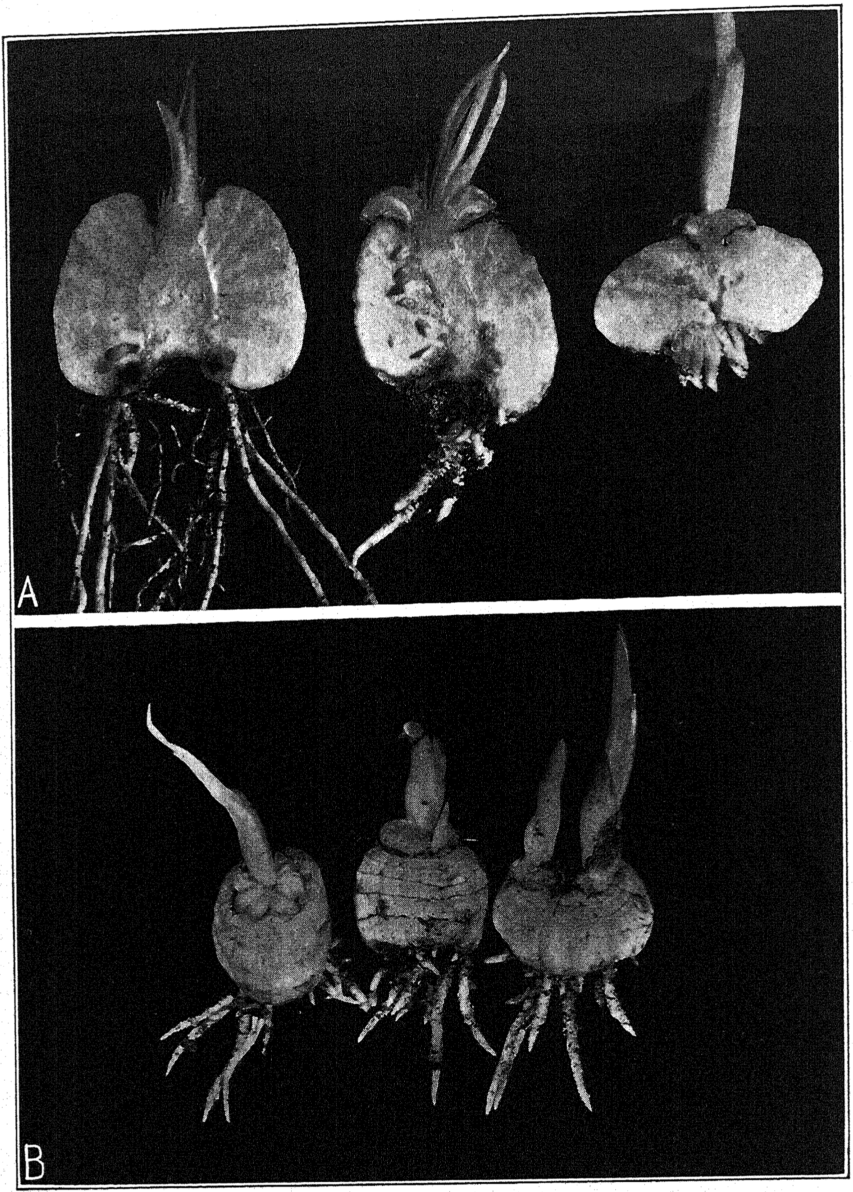

FIGURE 2. Gladiolus (Gold Eagle) corms treated with naphthaleneacetic acid, 100 mg. per liter, by soaking in the solution for 24 hours except one corm in (B) left, which was in solution while vacuum was applied for 30 minutes. Photographs were taken 14 days after treatment. (A) Longitudinal sections. Left, control; middle, median section through central axis of treated corms to show roots arising along the axis and contractile roots at top of axis; right, longitudinal section of side shoot. (B) Corms showing pronounced fasciation of contractile roots and retarded shoot development. Note the heavy roots at the base resembling normal contractile roots.

The experiments involving aeration with oxygen and carbon dioxide brought out some interesting contrasts. The corms aerated with oxygen while in treatment for 48 hours remained in good condition while the carbon dioxide set had a tendency to become spongy or rot after planting. This is in contrast with tubers of *Helianthus tuberosus* L. which disintegrated more quickly in oxygen aeration than in carbon dioxide. Aeration with oxygen of the tubers in water without the growth substance was detrimental while the carbon dioxide gave protection. The striking difference between the tuber and the corm in this respect cannot be understood with data at hand.

The greatest variation from normal growth was induced by treating dormant corms of White Butterfly. Figure 3 A shows roots growing from leaf scars all over the corm after treatment with 100 mg. per liter of indolebutyric acid for 48 hours while being aerated with oxygen gas. A few of the roots emerged some distance from the leaf scar but the majority followed the scars closely. This is similar to the responses of tubers of *Helianthus tuberosus* treated with naphthaleneacetic acid. In this case roots arose from practically every point on the tubers including the cut surface while controls grew only a few roots near bud points.

Figure 2 A shows roots arising along the central axis of corms soaked for 24 hours in naphthaleneacetic acid solution when normally roots arise only from the basal part. The same figure shows contractile roots developed in 14 days. When last examined, 40 days after treatment, the controls of this lot were just beginning to show contractile roots.

Figure 3 B shows roots arising in apparently unorganized fashion from the internal part of the corm. The corms were damaged from aeration with carbon dioxide for 48 hours while in the chemical solution and became somewhat spongy. Both naphthaleneacetic and indolebutyric acids produced this response but the latter was the less effective. Longitudinal sections through the fleshy part of the corms showed adventitious roots pointing in several directions. When these sections were examined with the low power of the microscope the adventitious roots could be seen as across and longitudinal sections. Many root primordia were scattered through the fleshy pulp. The basal part of the well-developed roots and the small primordia were always associated with vascular strands. Some of the vascular tissue was found running between the main strands of the leaf scars. These cross strands frequently gave rise to several root primordia in close proximity. The beginnings of fasciation could be seen where primordia originated near one another. These structures are illustrated in Figure 4.

Shoot development was definitely retarded with 100 mg. per liter of naphthaleneacetic acid and 200 mg. per liter of indolebutyric acid. With these concentrations the shield leaves had a tendency to become fleshy.

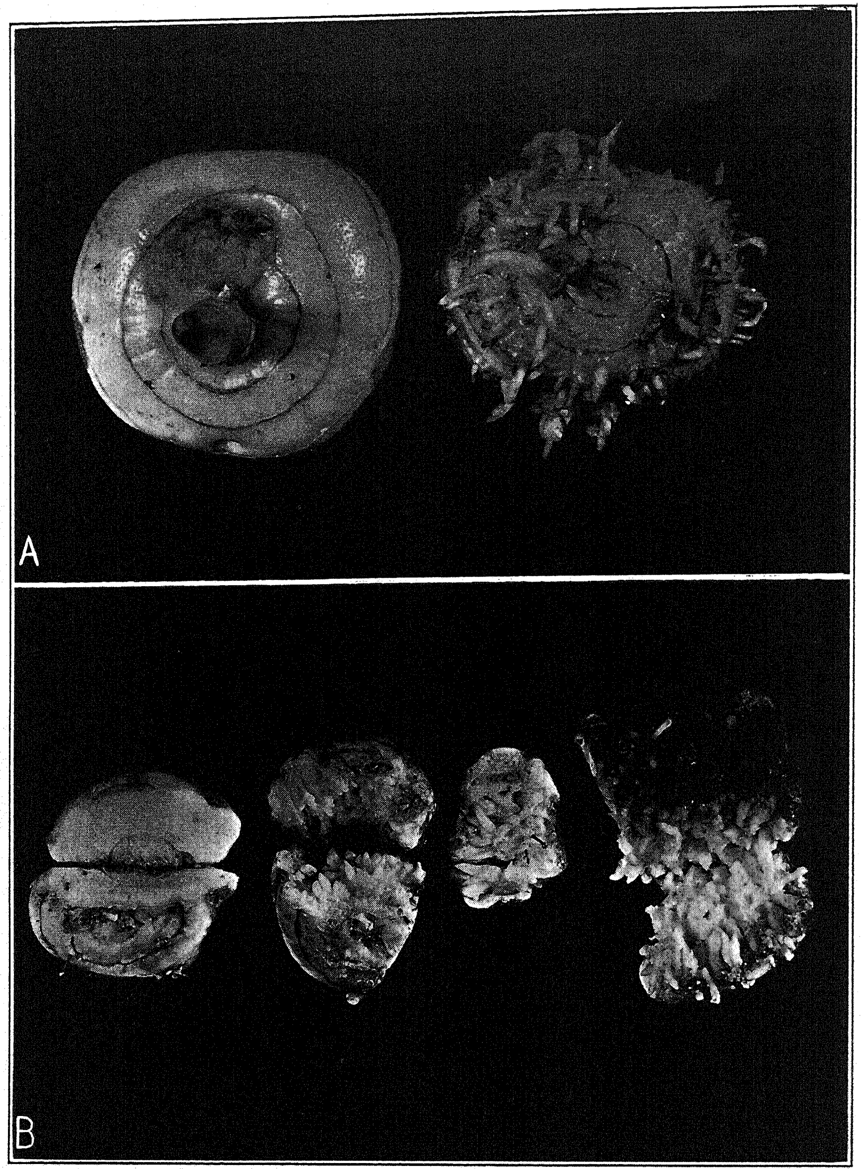

FIGURE 3. Gladiolus (White Butterfly) corms showing abnormal development of adventitious roots after treatment with growth substances. (A) Corms aerated with oxygen while in treatment for 48 hours. Left, water control; right, treated with indolebutyric acid, 100 mg. per liter. Note roots associated especially with leaf scars. Many roots also arose between leaf scars. (B) Corms aerated with carbon dioxide while in treatment for 48 hours. Left to right: control, all others treated with naphthaleneacetic acid, 100 mg. per liter. All corms were broken open from the top except small piece which was cut to show roots growing in all directions.

As the shoot developed the new leaves appeared normal (Figs. 1 A and 2 B).

Considerable speculation has been current as to the length of time the growth substances last after being taken into the tissues of plants. By use of a modification of the Winkler and Petersen (3) test we have been able to detect appreciable amounts of indole derivatives in developing roots and shoots of corms treated 24 days previously with indolebutyric acid (200 mg. per liter). Apparently the indole nucleus remained in the corm

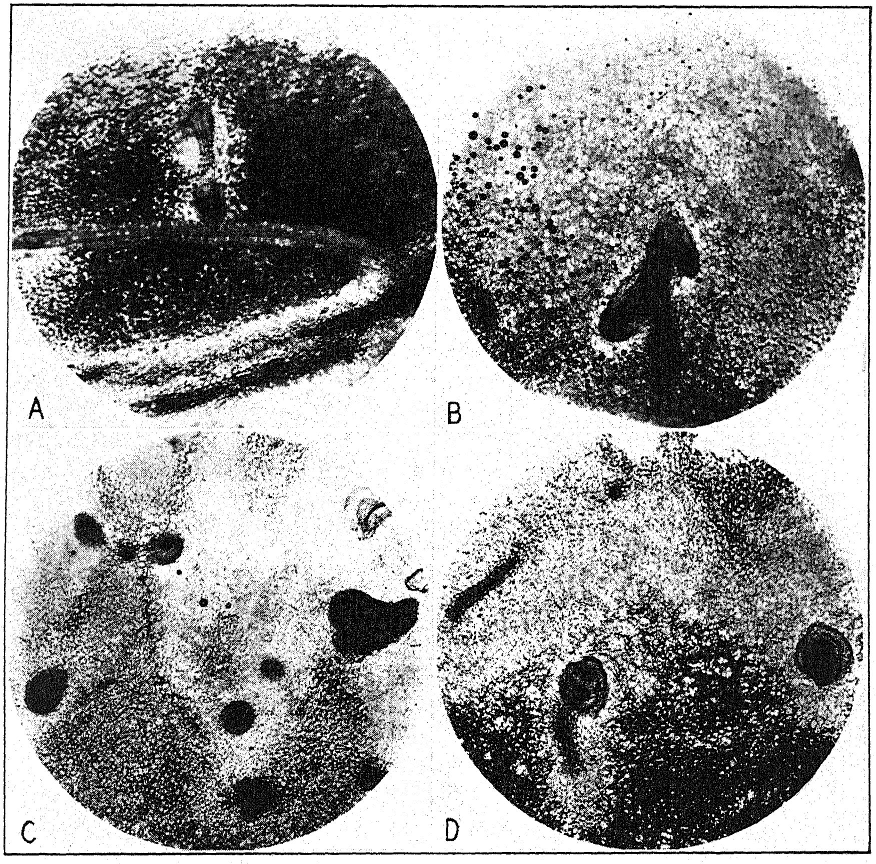

FIGURE 4. Photomicrographs of free hand longitudinal sections through the storage tissue of gladiolus corms (variety White Butterfly) treated with indolebutyric acid (100 mg./l.). The corms were immersed for 48 hrs. in the solution while being aerated with carbon dioxide gas. The photographs were taken 21 days after treatment of the corm. The sections were taken approximately one-half inch away from the central axis. Though the sections A, B, C, and D all came from approximately the same region of the corm, the roots appear in longitudinal and cross sections, D shows a cross section of a fasciated root.

and was translocated into the growing parts. It was not possible to identify the substance detected as indolebutyric acid, but the color obtained when the test was applied resembled that obtained with the pure compound. However, in the case of other plants and cuttings the applied growth substances were extracted in a physiologically active form and identified by the X-ray analysis (1, 4). A complete report of methods for detecting indole compounds and other growth substances in treated cuttings and plants will appear in another paper.

DISCUSSION

Some of the most striking results of these experiments were induction of adventitious roots throughout the storage organ while normally roots grow only around the basal axis, and the induction of contractile roots before the shoot developed. As a rule the contractile roots are associated with the newly-developing corm which becomes evident while the floral axis is growing. Corms treated with naphthaleneacetic acid produced contractile roots from the upper end of the central axis of the old corm. This is evident in the longitudinal sections shown in Figure 2 A. The same figure shows, also, that roots were induced along the central axis from tip to base. Those from the upper end, however, were always more fleshy than roots arising elsewhere (Fig. 2). This held for roots induced with both effective substances, but in all cases the roots induced with naphthaleneacetic acid were always more fleshy than those induced with indolebutyric acid. In fact the basal roots induced with the former were approximately as fleshy as the contractile roots induced by the latter (Fig. 1). This brings up the question of what constitutes the regulating influence of fleshy and fibrous roots all arising from the same central axis. Since the contractile roots can be grown without the presence of a new shoot when the right substance is supplied, this gives the impression that the regulating influence may be of a chemical nature. Under normal conditions the growing shoot probably furnishes this influence.

Considering the results illustrated in Figure 3 A, it is evident that adventitious roots can be induced with growth substances. The roots protruding from leaf scars and between leaf scars were not connected directly with the central axis. As determined by dissection, they were connected with vascular tissue which in turn connected the leaf scars to the central axis. In some cases vascular strands were found between the main vascular systems running from the leaf scar to the central axis. These also gave rise to roots (Fig. 4).

In the corms illustrated in Figure 3 B, roots seem to have arisen from all the vascular strands of the storage tissue and from the central axis. Before the picture was taken the corms were broken open from the top to show the mingled roots. A small piece was cut from the smaller corm to contrast with those broken. The roots were not growing in the same direction but were pointed up, down, and laterally. The fleshy part of the corm had become somewhat spongy. Such conditions were found only in dormant corms which were aerated with carbon dioxide for 48 hours while in the solutions. Comparable corms aerated during treatment with oxygen had roots protruding over the corm and the fleshy tissue had not become spongy.

It was surprising to find that a substance applied to the corm 24 days previously could be detected in the growing shoots and roots by the Winkler method. This fact militates against the assumption that indole growth substances are quickly disintegrated or used up by the tissues. It is also apparent that the growth substance can be translocated from the corms up the shoots and down into the roots.

As to the use of growth substances for practical purposes to force roots on corms slow to root, the prospects seem promising. Since the original tests we have not had corms which had lost entirely their capacity to root and that part of the problem still remains unanswered. At the present time, three varieties, treated in various ways, were set into the field to find if the induction of contractile roots ahead of the normal time will in any way aid the formation of new corms or flowering of the plants. Also corms were cut into several pieces before treatment to find if each piece can produce a healthy plant. This method might prove useful for rapid propagation of new varieties.

SUMMARY

Five varieties of gladiolus have been treated with three growth substances, naphthaleneacetic acid, indolebutyric acid, and indoleacetic acid, to stimulate root development and induce other responses.

The response of corms varied with the concentrations and the substances used. Naphthaleneacetic acid had a tendency to induce fleshy roots at the base of the corm resembling contractile roots normally formed at the base of new corms about flowering time.

Both indolebutyric acid and naphthaleneacetic acid were effective for inducing contractile roots within a few days after treatment whereas normally contractile roots arise at the base of newly developing corms as flower primordia are forming. The growth substances induced these roots from the upper end of old corms even in the absence of a shoot. The contractile roots induced by naphthaleneacetic acid had a tendency to be fasciated and fleshy, forming a complete ring around the new shoot. The higher concentrations of indolebutyric acid induced a few fasciated contractile roots but generally they resembled the normal type.

Under certain conditions corms were induced to form roots all over the storage organ though normally they form only around the basal part of the axis.

Corms aerated with oxygen withstood treatment much better than those aerated with carbon dioxide. The latter had a tendency to disintegrate but if the treatment was stopped in time it was the most effective for inducing adventitious roots throughout the storage tissue.

A substance giving the indole test was detected in growing roots and shoots 24 days after indolebutyric acid had been applied to the corms.

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HEATING AND LIGHTING GREENHOUSES WITH INTERMITTENT LIGHT

John M. Arthur and Edward K. Harvill

In a previous publication the construction and first year's operation of a new type of insulated greenhouse were discussed (4). The present report is concerned with the further operation of this greenhouse and in addition the operation of an ordinary greenhouse similarly lighted but partially heated by steam. These tests have been continued during four winter seasons in the insulated house and for three winter seasons in the ordinary lighted greenhouse. During three years' tests higher concentrations of carbon dioxide were maintained in the insulated greenhouse, while during the one remaining year no additional gas was used. The ordinary greenhouse has a much higher rate of air exchange with the external atmosphere and although this fact requires the addition of much more heat to maintain the desired growing temperature, at the same time it obviates the necessity of supplying additional carbon dioxide artificially in order to maintain greater growth and dry weight production.

Higher temperatures (62°–70° F.) were maintained during two seasons' growth while lower temperatures (60°–65° and 55°–60° F.) were maintained during the last growing seasons. Two methods of maintaining a desirable intermittency were used in the work. In most cases, thermostats were used to deliver sufficient lighting periods to maintain a definite temperature in the greenhouse. In several trial cases plants were grown under lamps operated by electric clock-driven mechanisms set to deliver constant intervals of lighting alternated with constant periods of darkness.

The present discussion therefore includes the effects in detail of these various factors on the growth and flowering of many different kinds of plants. In the later work an effort was made to study those plants known to have a high light and low temperature requirement, since thermostatically controlled lighting is well adapted for meeting such requirements.

INSULATED GREENHOUSE

While the construction, cost data, and first year of operation of the insulated greenhouse have been discussed in an earlier publication (4) two photographs (Fig. 1, A and B) and certain brief descriptive data are included herewith for those readers who do not have the previous publication available. Briefly, the house was built similar to a large refrigerator. The inside dimensions were 8 by 19 feet. The walls, floor, and part of the roof consisted of faces of sheet metal both inside and outside, nailed to 2 by 6-inch wooden structural members with the space between filled with

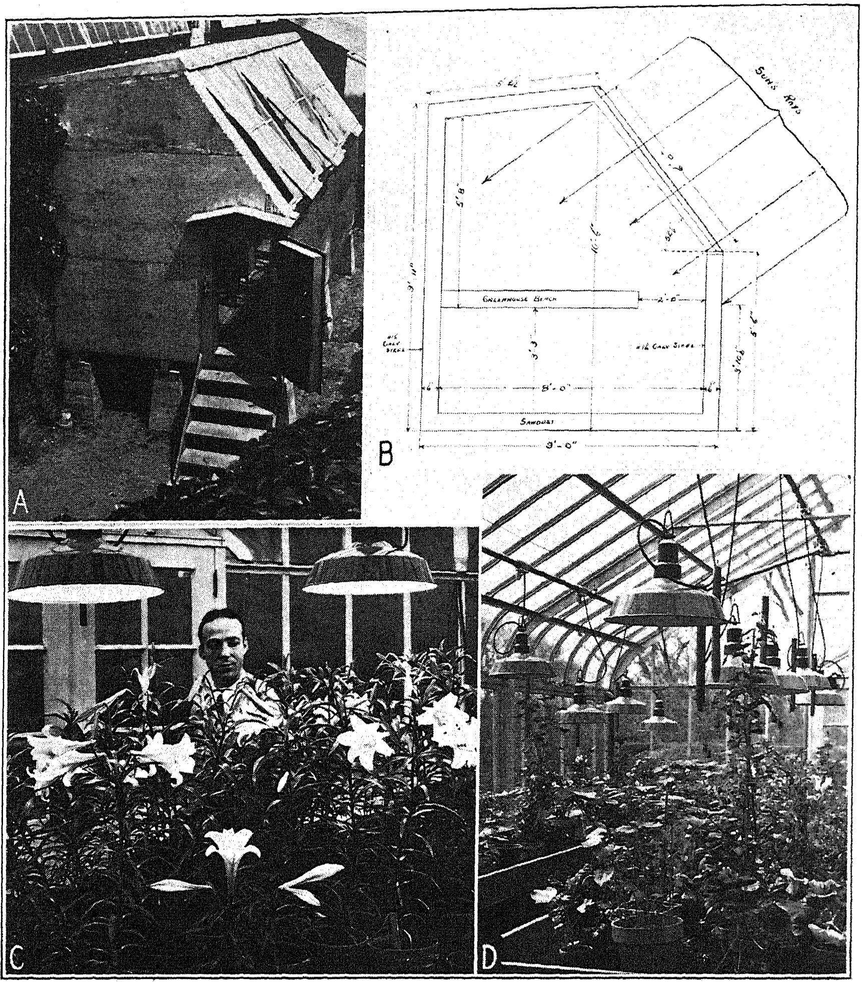

FIGURE 1. (A) Heat insulated greenhouse. (B) Cross sectional drawing through insulated greenhouse. (C) Lilies (December 24) grown with supplementary light each night, except short plants in foreground which were grown in control greenhouse. (D) View of center and south benches showing general lighting arrangement in ordinary type of greenhouse.

sawdust as the insulating material. While sawdust was used in the original construction and has now served the purpose for four years, it is believed that rock wool or similar insulating material which does not deteriorate upon wetting would be more satisfactory. Using this material, it is likely that the wall thickness could be decreased to 4 inches instead of 6 inches as originally provided. Since water condenses on the cold glass in winter and drips down continuously upon the floor where it accumulates until it can run out through two small floor drains (1/2-inch pipes) located toward either end of the building, it is believed necessary to cover the entire interior and especially the floor with sheet metal and to solder absolutely water-tight all joints which are at or near the floor level. The window section consists of eight separate, two-light, ordinary storm sash hinged at the top and arranged along the south face of the building. The sash can be propped open at the lower ends for ventilation. These windows are pitched at an angle of 52-1/2° so as to let in the maximum amount of winter sunlight with the least possible exposure of glass. The single, 6-foot growing bench was placed along the back or north wall of the building, leaving a walk 2 feet wide between the bench and the front or south wall. The inner walls above the bench were painted white so as to reflect as much light as possible down upon the growing plants. The house was lighted and heated, whenever sunlight failed, by means of two rows of 500-watt tungsten filament lamps spaced uniformly over the growing bench, each row consisting of five lamps. No other heat was supplied to the building. The lamps never burned in the daytime if the sun was shining, even if the outdoor temperature was at 0° F. During such periods of bright sunlight it was necessary to prop open the lower ends of one or two storm sash in order to keep the temperature down to 80° F. On cool, cloudy days and at night the lamps burned intermittently. The amount and time of burning were closely regulated by a small thermostat suspended above the center of the growing bench. This operated an electro-magnetic switch of sufficient capacity to turn all of the lamps on or off at the same time. Since the starting current on such banks of filament lamps greatly exceeds the normal operating current, it is important to install an electro-magnetic switch of sufficient capacity to take care of this overload.

ORDINARY TYPE GREENHOUSE, LIGHTED AND PARTIALLY HEATED BY LAMPS

In addition to the ten 500-watt lamps used for heating and lighting the insulated house, seventeen 500-watt lamps were used in some of the studies in a greenhouse of the ordinary type. This house was approximately 19 by 25 feet of the conventional ridge-roof, curved-eave type. One center bench 6.5 by 18 feet was illuminated by two rows of five each of the 500-watt lamps while a single 3-foot growing bench approximately 25 feet in length along the south side of the house was illuminated by seven 500-watt lamps. Figure 1 C shows some lilies grown on the center bench in this house and Figure 1 D a corner of the center bench and a part of the side bench. All 17 of these lamps were connected through the same electro-magnetic switch which was operated by the thermostat located in the center of the insulated greenhouse, so that all 27 lamps came on together, burned for the same time period, and all went off together. The only exception to this was during the last year's tests when only two of the seven 500-watt lamps along the south bench were operated on this general circuit, the other five being operated by special clock switching and other thermostatic switching devices to be described later. The ordinary type of house needed more heat than the lights supplied in order to maintain it at the same temperature as the insulated house. This additional amount of heat was supplied by means of steam heating coils operated by a thermostat and solenoid valve on the steam supply line. The operations of this valve were indicated by an electric clock which ran only when the steam valve was open, showing the number of hours of steam delivered to the greenhouse each 24-hour day. In addition, the number of operations of this valve per day could be further checked by the number of peaks on the daily temperature curve for each greenhouse. Temperatures for each house were continuously recorded by recording resistance thermometers. A third greenhouse of the ordinary type was maintained at the same temperature by means of thermostatically controlled steam lines. This house had no additional light and served as a control house to determine the normal amount of growth at this temperature under the sunlight which is available in this region during the winter months.

PERIOD AND AMOUNT OF ILLUMINATION PER DAY

It should be recalled that the amount of light received by plants in the heat insulated greenhouse was always the same as that received on the center bench of the ordinary type greenhouse since both sets of lamps were operated by the same electro-magnetic switch. The insulated greenhouse required 13 to 18 kilowatts per day as an average figure for the entire winter season depending upon the night temperature which is to be maintained inside the house. With current at 2¢ per kilowatt, it costs 26¢ to 36¢ per average day to heat and light the house during the winter months. The growing bench so lighted is 6 by 19 feet. The lower of the cost figures obtains when a low night temperature of 55°–60° F. is maintained. At this low temperature range the lamps burn for a period of 10 to 12 minutes alternating with an off or dark period of 12 to 30 minutes, depending upon the rate of heat loss inside the greenhouse. The rate of heat loss depends in turn upon the outside temperature. At 27° F. the off period is about 22 minutes, while at 9° F. the off period is only 12 minutes. In the years 1937 and 1938 the average daily burn was 3.4 hours per 24-hour day during the coldest months, December, January, and February, and 1.6 hours during the months of November and March. The lamps never burned during the daytime if the sun was shining even when outside temperatures were near 0° F. On cloudy days and at night the lamps burned intermittently. All lamps were suspended by means of a cord, pulley, and sash weight arrangement so that they could be raised and lowered easily. The tips of the lamps were kept at about 18 inches to 2 feet above the tips of the plants.

TEMPERATURE

In this work it has been observed frequently that cool night temperatures are much more important than day temperatures in case of those plants requiring cool temperatures for flowering. This is indeed fortunate, for night temperatures in greenhouses are much easier to control than day temperatures where the amount of solar radiation reaching the house varies from minute to minute. During the four winter growing seasons represented in this report the following temperature ranges were maintained each night:

1934–35 temperature range 62°–68° F.
1935–36 " " 68°–70° F.
1936–37 " " 60°–65° F.
1937–38 " " 55°–60° F.

During the last season a fourth greenhouse which was used for lilies, strawberries, and other plants was kept at 75° each night. This house had a single 500-watt lamp in one corner which was burned for 6 hours continuously each night. While some effort was made to keep the day temperatures similar to night temperatures, the day temperature average was always higher than the night temperature and always much more variable.

OTHER METHODS OF TIMING INTERMITTENCY OF LAMPS

Small thermostat. During the last two winter seasons, other methods of producing intermittent burning of groups of two or three 500-watt lamps were used in comparison with the method outlined above. These tests were made on the south bench of the same greenhouse as was used in the other lighting tests. In one of these a small 25-ampere thermostat similar to that suitable for operating soil heating cable was used. Two 500-watt lamps were operated on this circuit and the thermostat was suspended directly beneath one of them. The method is illustrated in the text (Fig. 2 A). This appeared to work satisfactorily where no more than two or at most three 500-watt lamps were used. Its main difficulty is that the lamps tend to burn for a shorter interval than is desirable. Since the thermostat and lamps are comparatively inexpensive this method has been adapted recently to the lighting and heating of bay-windows or small divisions of sunporches where only a few plants are to be grown by the householder. A small inexpensive electric clock can be placed in the circuit to indicate the total number of hours of burn of the lamps and the thermostat should be adjusted to give a total burn of 3 to 6 hours each night, and a lighting interval of at least 10 minutes.

Clock mechanisms. In addition, small electrically driven clocks were used to drive a gear and cam mechanism which threw a mercury switch of sufficient capacity to operate three 500-watt lamps. Since the construction of a satisfactory clock switching arrangement for this work is attended with no little difficulty, a brief description of it is included herewith. The first clocks made for this purpose were of the commutator type with metal and fiber sectors alternating on a contacting drum which was rotated by the electric clock. When contact was made on the metal sectors a relay operated to turn the lights on. This proved eventually to be wholly unsatisfactory and had to be discarded. Finally a thin steel wheel about 2 inches in diameter upon which two cams were mounted was used. The

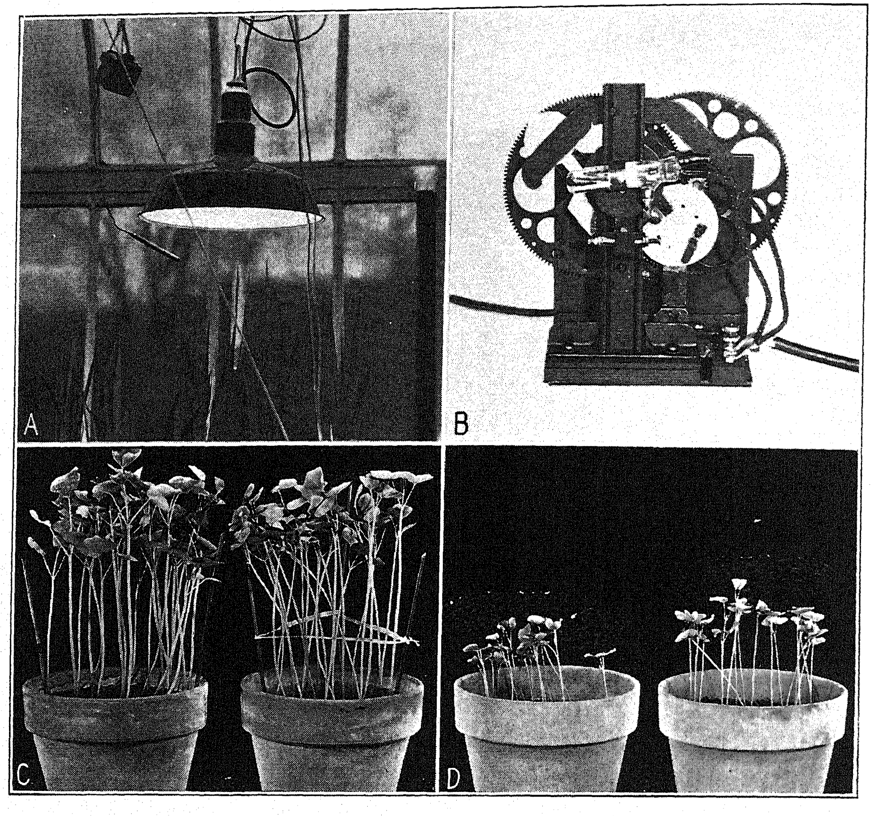

FIGURE 2. (A) A method of operating two 500-watt lamps directly on a soil heating cable thermostat so that they burn intermittently. (B) Clock mechanism switch with cams set for timing of lamps, 2-1/3 hours on, alternating with 1-1/2 hours off interval. (C) Buckwheat plants grown under all artificial light at 68°–72° F., (left) continuous illumination, (right) intermittent illumination, 5 seconds on, alternated with 5 seconds off. (D) same as (C) except at a temperature of 95°–100° F.

cams were separated at the correct angular distance to throw an inverted pendulum first to the right and then back to the left against rubber bumping posts. A mercury switch was mounted on the axle of the inverted pendulum and tipped back and forth with it, alternately making and breaking the lamp circuit. The wheel carrying the cams was driven by an electric clock and an appropriate train of gears to produce the required timing. A photograph of this clock switch is shown in Figure 2 B. This dead fall type of switch is positive both ways in its action and never failed to operate during the entire winter season in which it was used. Two of these switching clocks were used in the last tests. One gave a period of 6 minutes, 8 seconds on for three 500-watt lamps, alternated with an off or dark period of 4 minutes, 24 seconds, from 6:00 p.m. to 6:00 a.m. each night. The other gave a period of 2 hours and 20 minutes on, alternated with an off or dark period of 1 hour and 31 minutes for one 500-watt and two capillary mercury vapor lamps (type H-3) from 6:00 p.m. to 6:00 a.m. each night. The total number of hours of burn in each case was approximately the same. The average per night for 14 nights for the shorter interval lamps was 7.06 hours as compared with 7.14 hours for the longer interval lamps. These figures are those actually recorded by two independent electric clocks which operated only when the lights came on and are not calculated from the summations of the off and on intervals of the clock switches. The night watchman plugged in the switching clocks each evening at 6:00 o'clock and the clocks started from the same position in which they stopped when the plugs were pulled out previously at 6:00 o'clock on the morning of the same day.

RESULTS OF TESTS WITH LIGHTING FREQUENCY TIMED BY CLOCK MECHANISMS

During the latter part of March some dry weight production tests with buckwheat plants growing in eight-inch pots were made under the long interval as compared with the short interval lamps. The seedlings were grown in a warm greenhouse (75° F.) until they were about two inches high. Two uniform pots of seedlings were then placed under each 500-watt lamp and at the same distance from the lamp in each case. In addition two similar pots of seedlings were grown in another greenhouse without additional light at the same night temperature (55°–60° F.), and two more pots of seedlings were grown in another greenhouse at a night temperature of 75° F. The dry weights produced after a two weeks' growth period are shown in Table I.

It is seen from the above results that there is little difference in dry weight of buckwheat produced by short intervals of lighting (6 minutes, 8 seconds) and long intervals (2 hours, 20 minutes) as a supplement to sunlight provided that the total number of hours per night is approximately the same. This fact is important when considering the advantages of controlling the amount of supplementary lighting by means of a thermostat since the duration of the light and dark intervals varies with the rate of heat loss and this in turn varies with outside temperatures. The "forcing" or heating effect on hyacinth and lily buds was observed to be much greater under the longer interval, however. Flowers developed more rapidly from buds and produced shorter stems under the longer lighting periods and subsequently faded more rapidly. This might be expected since it is well known that heat as such has this effect and it is reasonable to assume greater heating effects on longer lighting intervals.

TABLE I

DRY WEIGHT PRODUCTION OF BUCKWHEAT IN GREENHOUSE WITH INTERMITTENT LIGHT

Lighting interval	No. of plants	Total dry weight, g.	Dry weight per plant, g.
Sunlight plus 7.06 hours of light each night at intervals of 6 min., 8 sec. on, alternated with 4 min., 24 sec. off	51	21.506	0.422
Sunlight plus 7.14 hours of light each night at intervals of 2 hr., 20 min. on, alternated with 1 hr., 31 min. off	47	19.704	0.419
Sunlight only. Same temperature as above, 55°–60° F. at night	45	11.416	0.254
Sunlight only. Temp. 75° F. at night	47	10.032	0.213

Several other similar tests were made the year before with shorter light intervals of 5 seconds on, alternating with 5 seconds off. Three of these tests were made under all artificial light in the constant light room (1) where temperature was accurately controlled. Three separate tests at three different temperatures were made using four pots of buckwheat plants under intermittent light on one side of a sheet metal baffle and four similar pots at the same time on the other side of the baffle under continuous light conditions. A single 500-watt, 110-volt lamp, operated on a 120-volt line was used in each case. Intensities were adjusted to the same value in each test. The average distance from the lamp to the soil was 26 inches, and the plants were grown for 10 days. A summary of the dry weight data is shown in Table II.

Photographs of plants grown in two of the tests under all artificial light are shown in Figure 2, C and D. It is evident that development of the plants was greatly restricted by the higher temperatures (95°–100°, Fig. 2 D). Plants exposed to intermittent light at a high temperature for only one-half the time (5 seconds on, alternating with 5 seconds off) produced almost as much dry weight as those exposed continuously. At the lowest temperature (68°–72° F.) plants grown under intermittent light produced, on the average, slightly more than one-half the dry weight of those exposed continuously. At the intermediate temperature (85°–90° F.) plants receiving intermittent light produced less than one-half the dry weight of those exposed continuously. While the values show considerable individual variation it is evident that at the lower temperatures those plants lighted intermittently for only one-half the time produce approximately one-half the dry weight of those lighted continuously. The failure of this relationship to hold at the highest temperature is believed due to a heat injury effect on plants exposed continuously, while plants exposed intermittently have brief periods of rest and opportunity for heat loss during the dark periods. It is apparent from the dry weights and the photographs that all plants, whether exposed either intermittently or continuously, were held back by the higher temperatures.

TABLE II

DRY WEIGHT PRODUCTION OF BUCKWHEAT PLANTS UNDER ARTIFICIAL LIGHT

Temperature	Intermittent*		Continuous	
	No. of plants	Av. wt. per plant, g.	No. of plants	Av. wt. per plant, g.
68°–72° F.	29 25 22 24	0.078 0.083 0.075 0.074	21 20 24 22	0.141 0.140 0.123 0.155
Total av.	100	0.077	87	0.140
85°–90° F.	19 24 21 17	0.045 0.041 0.047 0.049	15 17 17 20	0.122 0.086 0.112 0.128
Total av.	81	0.046	69	0.112
95°–100° F.	15 19 19 18	0.049 0.038 0.042 0.035	15 15 14 17	0.054 0.051 0.046 0.047
Total av.	71	0.041	61	0.050

* Five seconds on, alternated with 5 seconds off.

RESULTS OF GROWTH TESTS IN GREENHOUSES WHERE PERIOD OF INTERMITTENT LIGHTING WAS DETERMINED BY THERMOSTATIC ACTION

Since this method was found to be the best arrangement for supplementary lighting both in the ordinary greenhouse and in the heat insulated type a more detailed discussion of the plants grown and the results obtained are given. The following plants were grown during two or more of the past four winter seasons in which this method of supplementary lighting has been used:

Antirrhinum majus L. (snapdragon, Giant Mixed)
Begonia rex L.
Begonia semperflorens Link & Otto
Calceolaria sp. (hybrids)
Cymbidium insigne Rolfe (orchid)
Dianthus caryophyllus (carnation)
Euphorbia pulcherrima Willd. (poinsettia)
Fagopyrum esculentum Moench. (buckwheat)
Fragaria chiloensis Duchesne (Dorsett strawberry)
Fuchsia hybrida Voss
Gardenia jasminoides Ellis
Gerbera jamesoni Bolus
Gladiolus childsii Hort. (Mrs. F. C. Peters)
Gladiolus gandavensis Van Houtte (A. E. Amos, Red Gauntlet, Loch Nagar)
Gladiolus lemonei Hort. (Daintiness)
Gladiolus primulinus Baker (White Butterfly, Queen of Bremen)
Hyacinthus orientalis L. (L'Innocence, Bismarck, Queen of the Blues, Grand Maitre respond to light only after 3 months' growth in cold dark room.)
Lathyrus odoratus L. (sweet peas, mixed)
Lilium longiflorum Thunb. (Erabu, Giganteum)
Lilium longiflorum Thunb. var. *eximium* Nichols (*L. harrisii*)
Lilium tenuifolium Fisch.
Matthiola incana var. *annua* Voss (stocks, hybrids)
Papaver orientale L. (oriental poppy responds to light only after cold treatment.)
Pelargonium zonale Willd.
Petunia hybrida Hort.
Primula sp. (hybrids)
Rose (Talisman)
Senecio cruentus DC. (cineraria, Giant Exhibition)
Solanum pseudocapsicum L. (Jerusalem cherry)

All of the above plants flowered well under such supplementary lighting except cineraria and poinsettia. Some confusion has appeared in the literature recently in assigning some plants such as cineraria to both short and long day categories. Thus Post (15) and others using lower intensities of supplemental lighting have assigned cineraria to the list of plants responding to supplemental light, while Arthur and Porter (4) found that both cineraria and poinsettia should be classed as short day plants. Recently Eguchi (7) reported that both cineraria and strawberry belong to a group of plants which requires a short day for flower bud differentiation and a long day to develop buds and produce flowers. In the work of Post the plants were placed under lights late in the season to bring them into flower, no doubt after bud differentiation had occurred, so that long days were found favorable, while in the work of Arthur and Porter and in the present work the plants were lighted each night during their entire growing period with high intensity illumination. When lighted in this way only 2 plants out of 22 flowered by February 26, while all 24 plants in the control house, without additional light, flowered by this date. Many of the plants in the lighted greenhouse came into flower in April, at a time when little or no light was being used each night. (The average number of hours of light per night for March was 1.3 as compared with 0.9 hour for April.)

The main results with various species will now be discussed in alphabetical order.

Begonia. Both large flowering tuberous rooted and small flowering varieties were grown. These plants were favored by a night temperature of 65°–70° F. Both the size of the plant and the number of flowers were greatly increased by supplementary lighting. The plants respond well to continuous illumination from sodium vapor and capillary mercury lamps as has been noted previously (3).

Buckwheat. Pots of buckwheat were grown and harvested at frequent intervals to determine the dry weight of plant tissue produced per unit of time under the different lighting conditions. It was found by observation upon many kinds of plants that growth and development in the insulated greenhouse without additional carbon dioxide was slower than in the ordinary greenhouse where similar lighting was applied. The results of tests with buckwheat shown in Table III indicate this. During the first period from November 7 to December 11 little light was used and there was only a slight increase in dry weight produced in the two lighted greenhouses as compared with the control or non-lighted house. During the cold period (December 11 to January 14) the dry weight production in the control was 0.14 gram per plant as compared with 0.52 for the ordinary lighted greenhouse and 0.40 for the insulated greenhouse. Plants growing under 300-watt lamps in the ordinary greenhouse had an intermediate dry weight of 0.34 gram. During the period January 14 to March 4 the dry weight produced in the control greenhouse gained rapidly but did not equal either of the lighted houses. From March 4 to April 14 the control plants grew better than those in the insulated house but did not equal those in the ordinary greenhouse. This failure of the insulated house to equal the control greenhouse in the spring and summer months is due to the design of the roof (shown in Fig. 1 B) which admits winter sunlight

TABLE III

DRY WEIGHTS OF BUCKWHEAT PLANTS PRODUCED IN EACH TYPE OF GREENHOUSE DURING PERIODS INDICATED. SEASON OF 1935–1936

	No. of plants	Av. dry weight per plant, g.
A. Grown from November 7 to December 11		
Control greenhouse	44	0.22
Greenhouse with 500-watt lamps	38	0.27
Insulated greenhouse with 500-watt lamps	47	0.24
B. Grown from December 11 to January 14		
Control greenhouse	40	0.14
Greenhouse with 500-watt lamps	30	0.52
“ “ 300-watt “	30	0.34
Insulated greenhouse with 500-watt lamps	64	0.40
C. Grown from January 14 to March 4		
Control greenhouse, sample (1)	20	1.10
“ “ “ (2)	34	0.90
Greenhouse with 500-watt lamps, sample (1)	27	3.06
“ “ “ “ “ (2)	18	2.72
Insulated greenhouse with 500-watt lamps, sample (1)	20	1.33
“ “ “ “ “ “ (2)	18	1.40
D. Grown from March 4 to April 14		
Control greenhouse, sample (1)	20	1.32
“ “ “ (2)	20	1.43
Greenhouse with 500-watt lamps, sample (1)	32	1.92
“ “ “ “ “ (2)	26	1.68
Insulated greenhouse with 500-watt lamps, sample (1)	16	0.56
“ “ “ “ “ “ (2)	20	0.53

very efficiently but transmits much less light in spring, summer, and fall. In fact, much of the house receives only skylight during the summer, only the front part of the growing bench receiving direct sunlight. The results of this study show that the insulated greenhouse using the same amount of artificial light is not as effective in dry weight production as the ordinary greenhouse with artificial light. The difference is believed due mainly to the low rate of air exchange as compared with the ordinary greenhouse and its attendant restriction of carbon dioxide supply. In all other winter season tests an additional supply of carbon dioxide has been available in the insulated greenhouse and there has been much less difference in growth and dry weight produced between the two lighted houses. The methods used for supplying carbon dioxide to the insulated greenhouse will be discussed later.

Calceolaria. In an earlier publication (4) this plant was reported as responding well to supplementary light. The chief difficulty was an un-

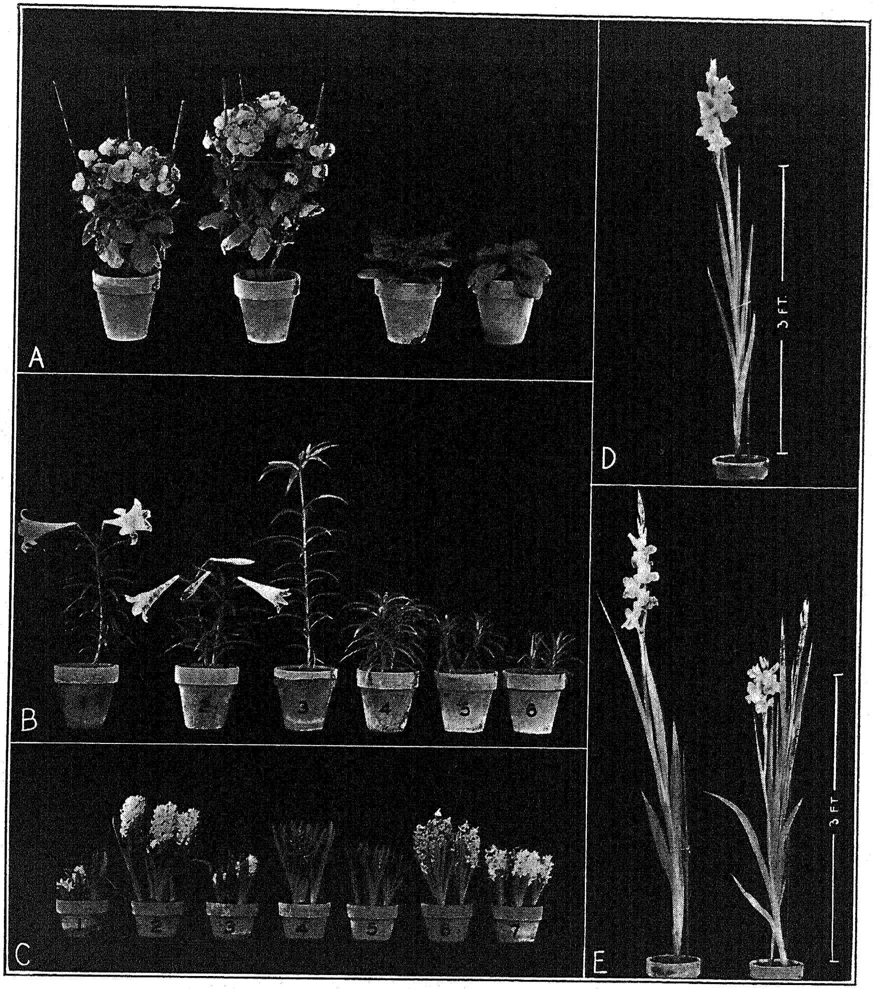

FIGURE 3. (A) Calceolaria, two at left grown with intermittent light each night, two at right control greenhouse without additional light. (B) *L. harrisii*, bulbs planted September 17, photographed December 8: (1) short interval intermittent light, 55°–60° F.; (2) no extra light, night temperature 75° F.; (3) intermittent light, thermostat, general lighting circuit 55°–60° F.; (4) no light, same temperature as (3); (5) cold treatment until November 9, then 55°–60° F. greenhouse; (6) cold room at night, greenhouse during daytime. (C) Hyacinths, planted October 1, photographed January 24, left to right: (1) temperature 55°–60° F., short period intermittent light (clock mechanism) no cold temperature treatment; (2) cold treatment until December 22 then long period intermittent light (clock mechanism), 55°–60° F.; (3) cold room each night and greenhouse during daytime until January 9, then light treatment same as (2); (4) cold treatment until January 7, then light treatment as in (2); (5) cold treatment until January 12, then short period intermittent light (clock mechanism); (6) and (7) cold treatment until January 7, then 75° F. greenhouse with 6 hours continuous light each night. (D) Gladiolus var. A. E. Amos, and (E) var. Red Gauntlet grown with intermittent light.

desirable spindling type of growth of the flower stalks. This spindling habit was found to be due to the higher night temperatures (68° F.). In Figure 3 A are shown plants grown at 60°–65° F. night temperature in 1936–1937 which are much more sturdy and desirable in type. During the last season (1937–1938) plants were grown at a still lower night temperature of 55°–60° F. producing an even more sturdy and dense type of growth, although slightly slower to attain the flowering stage. Bailey (5, v. 1, p. 625) recommends for calceolaria a night temperature of 40° F., allowing a 10° or 15° rise during the day. Since temperature requirements are in general slightly higher when artificial light is used probably the 55°–60° range will prove most desirable. The original home of many of the calceolarias was the Andes Mountains of Chile and Peru where the plants were no doubt adapted to high light intensity, long days, and lower temperatures. The modern hybrids appear to be still well adapted to such climatic factors, and can be well grown with supplementary high intensity light where thermostatic control is provided to control the accompanying increase in temperature.

Carnation. The carnation also requires a low temperature for best growth. In work published earlier (1, p. 475) it was reported that this plant did not respond to light and carbon dioxide at a high temperature (78° F.). In the last season's tests at night temperatures of 55°–60° F., carnation plants flowered best in the lighted houses. The average cut of flowers per pot of plants from January 24 until May 14 was 2.5 for the control greenhouse, 4.6 from the supplementary lighted, and 4.6 from the insulated greenhouse. There were 45 pots of plants in the control, 21 in the lighted greenhouse, and 12 in the insulated house. Plants in both of the lighted greenhouses reached maximum production early in February while those in the control house did not produce well until April. The foliage and new shoot production held up best in the control plants as the spring season progressed. While the additional light greatly aided flower bud and flower production, foliage growth seemed to be better in the control house. All plants were received from a grower on September 29, in poor condition. They were potted at once in 7-inch pots, but were slow to recover and start growth again. For this reason they were late in coming into flower production in all houses.

Gardenia. It has been noted in an earlier publication (2) that gardenia responds well to artificial light, but that night temperatures of 60°–65° F. are favorable for bud development. During the past season these plants were grown at night temperatures of 55°–60° with and without additional light. Both the smaller and larger (Hadley) flowering varieties were used in the tests. Bud development and flowering again proceeded well at the lower temperature where plants were illuminated for a few hours at night but leaf color toward the end of the winter season was much paler at this lower temperature. Jones (11) has recently shown that chlorosis can be avoided by carrying the soil temperature above 65° independently of the air temperature. He has also reported that plants transferred from a high temperature (90° F.) to a low soil temperature of 50° F. produce a large number of flower buds. This confirms the fact brought out in an earlier publication (2) that cool nights below 65° favor bud development in gardenia while temperatures above 70° favor good growth but little or no bud development. It has also been observed that plants held at the lower temperature until buds have developed can be returned to a higher temperature where the buds will continue to develop rapidly into flowers. Such plants have very dark green foliage but generally flowers are smaller and do not appear to last as long when cut.

Gladiolus. The corms for these plants were planted on October 5. A few 5-inch pots were planted with single corms. In most of the tests two corms were planted in each 7-inch pot. Many of the large-flowered varieties were included at the request of Forman T. McLean who was interested in crossing these with small, sweet-scented varieties (14) which normally flower only in the late winter months. The large-flowered varieties had failed to flower in the ordinary greenhouse during the winter months without additional light. In general only a few varieties of gladiolus will flower in the late winter in the greenhouse in this locality, although several varieties are shipped in from Florida where they are grown out-of-doors.

It was found that the following varieties flowered well under the general intermittent lighting conditions already described (thermostatically operated from insulated house): Daintiness, Red Gauntlet, A. E. Amos, White Butterfly, Queen of Bremen, and Loch Nagar. The first flower was produced on Daintiness on January 28; the second by Red Gauntlet on February 3 (Fig. 3, E and D). The others followed at slightly later dates. Red Gauntlet produced a flower spike 56-1/2 inches tall and several others of the large flowering types were equally tall. Several spikes produced 12 or more flowers and all set large quantities of viable seed when cross pollinated. The night temperature was 55°–60° F. When two plants are grown in a single 7-inch pot the total cost for current for a single spike of gladiolus is approximately 5¢ if this method of lighting is continued until the last of January. If the corms were planted a month earlier and could be brought out into flower one month earlier, approximately one-third of the lighting costs would be saved. This method of high intensity illumination applied intermittently appears therefore not too costly to be practical for the flower grower who is producing the more valuable varieties of gladiolus. This is especially true when it is considered that a large part of the heating load is carried by the lighting arrangement in the ordinary type of greenhouse and all of the heating load in the heat insulated house. Gladiolus was not, however, included among the plants grown in the heat insulated house during the last winter season, but since the lighting conditions were identical it is believed that a similar result would be obtained.

Hyacinth. Following the method of Slogteren (16), it had been determined the year previously that hyacinths required a low temperature treatment before they would respond to light. Three or four bulbs of each of the following four varieties were planted in soil in 6-inch bulb pots on October 1: L'Innocence, Bismarck, Grand Maitre, and Queen of the Blues. A few pots were placed at once in the lighted greenhouses at a night temperature of 55°–60° F. Several of the other pots were stored in a cold dark room at 50° F. Another set of pots was placed on a moving schedule of cold dark room (50° F.) at night and a greenhouse during the day at about 70° F. until January 9 after which time they were held continuously in the lighted greenhouse. Many plants had grown to a height of one inch above the soil level by December 14. One set was removed from the cold room on December 22, others on January 7, 9, and 12, and placed in the lighted greenhouses. A representative set of the plants was photographed on January 24 and is shown as Figure 3 C. All plants placed in the greenhouses at the start without any cold treatment appeared much the same as Figure 3 C, plant 1. Neither the leaves nor flower stalks grew normally. All plants removed from continuous cold dark room treatment on or after December 22 continued development. The flowers following this treatment could be brought out very quickly by placing them in a 70°–75° night temperature greenhouse and lighting for 6 hours continuously each night (Fig. 3 C, plants 6 and 7) or could be brought out more slowly in a lower temperature greenhouse (55°–60°) with intermittent light (Fig. 3 C, plants 2, 4, and 5). Those developed rapidly at the higher temperature were not so tall nor so well developed as those at the lower temperature with intermittent light, nor did the flowers last as long. The cold night treatment, alternated with a warm greenhouse during the day brought only partial recovery as shown in Figure 3 C, plant 3. Such plants could not be exposed unduly to heat and light. Those plants receiving the lighting schedule of 2-1/3 hours on, alternated with 1-1/2 hours off throughout the night at a temperature of 55°–60° did not grow normally although they were much better than Figure 3 C, plant 1, which received no cold treatment. Several of those plants under less rigorous intermittent light in the insulated house finally grew almost to normal height, flowering several weeks later. Perfect development of flowers following such incomplete cold treatments depended mainly upon variety; L'Innocence and Bismarck responded better in this respect than Grand Maitre or Queen of the Blues. It is evident from these responses that when hyacinth plants have had a sufficient exposure to low temperature, flower stalk and full leaf development follow rapidly and additional light and higher temperatures increase the rate of the process. Plants 6 and 7 in Figure 3 C were brought into full flower in 14 days after removal from the cold dark room. The cold storage period was 98 days. Plant 4 in this figure was brought out of cold storage on the same day (January 7) but exposed to a lower night temperature (55°–60°) and general intermittent lighting (thermostat regulation). These flowered on January 28, or 21 days' light exposure. Plant 5 in this figure removed from the cold room on January 12 was in full flower on January 28 (6 minutes, 8 seconds on, and 4 minutes, 24 seconds off schedule). Both plants 4 and 5 flowered normally. Plant 2 was brought from the cold dark room on December 22 and developed slowly by exposure to the capillary mercury lamp on a lighting schedule of 2-1/3 hours on, alternating with 1-1/2 hours off each night over a period of 33 nights following 83 days in cold storage. It is not known whether plants removed as early as December 22 would develop fully when exposed to the more rigorous treatment of high temperature and 6 hours of continuous lighting each night. Only lower temperatures and intermittent lighting were used on the plants removed from the cold room on this date. Since these plants required 33 days' exposure to light to reach the full flowering stage as compared with 21 days for those removed from the cold room on January 7, it is apparent that the cold treatment was not complete by December 22, or 82 days.

Probably the best treatment for hyacinths is to plant them in soil and store them in darkness for a period of at least three months at a temperature of 50° F. Following this the plants can be placed in a greenhouse and given intermittent light and a temperature which will force them rapidly or slowly in line with the requirements of both the market and the grower. Hyacinths placed in storage in June have been brought into flower on the last day of September by this treatment during the present study. It is probable therefore that they can be brought out at any time desired by the grower. The plants require greenhouse space for a very short time (3 or 4 weeks), spending most of their life (3 months) in a cold storage room. It is evident from the results above that hyacinths will not respond to light unless the cold treatment has been given.

Lily. The lilies were grown to determine the possible response to artificial light and temperature as regards the following: (1) Could two crops be grown in a single winter season in the greenhouse? (2) Is there any relation between rate of development and number of flowers produced? (3) Is cool temperature treatment an aid in development of flower axis as in hyacinth? Bulbs of *L. harrisii* were furnished by Mr. John Vaughan. They came from southern Japan and were planted in pots of soil on September 17 after a dry storage period of probably 7 to 8 months. A set of the plants was kept continuously in the dark in a 50° F. room. Another set was placed in the lighted greenhouse (night temperature 55°–60° F.), and a third set placed in an unlighted greenhouse at the same temperature. A fourth set was grown in the insulated greenhouse. On October 13 some of the plants were taken out of the cold dark room and given an exposure in the greenhouse each day and returned to the cold room each night. In addition some plants were transferred to a 75° F. greenhouse (night temperature) as soon as they had appeared above the soil. A part of these were given 6 hours' continuous light each night at this high temperature and others were not lighted.

The first flowers appeared on December 7 both in the 75° house and in the greenhouse at a lower temperature (55°–60° at night) exposed to intermittent light (6 minutes, 8 seconds on, alternated with 4 minutes, 20 seconds off—a total of about 7 hours per night). A photograph of representative plants was taken on December 8 and is shown as Figure 3 B. The shortest lilies were produced at high temperature (75° at night) without light (Fig. 3 B, plant 2); the tallest at a temperature of 55°–60° at night with the general intermittent lighting regulated by a thermostat (Fig. 3 B, plant 3). All of those having a continuous cold treatment or cold treatment each night were greatly delayed as to flowering without any compensating gain in size of plant or size of flower as shown in Figure 3 B, plants 5 and 6. Figure 3 B, plant 4, was grown continuously at a 55°–60° night temperature in a greenhouse without lights. The appearance of some of these plants grown with lights on thermostatic control at 55°–60° night temperature compared with controls from the unlighted house is shown in Figures 4 A and 1 C. The photographs were taken December 24, 98 days after planting. The average height of those grown without lights was 12 inches as compared with 32 inches under intermittent light. The set of plants kept continuously in the cold dark room from September 17 to November 9, then grown in the 55°–60° greenhouse either with or without lights, did not flower until February 8, or 144 days after planting. Following such cold treatment, lighting was much less effective in hastening flowering. Plants given cold nights and warm days until January 10 flowered the second week in February but were dwarfed considerably by the treatment. Plants in the insulated greenhouse flowered early in January. While plants could be increased greatly in height by transferring them at any time before flowering to the lighted house, the greatest height and best growth was attained only by those grown continuously under intermittent light regulated by thermostats. Plants grown under intermittent light regulated by clock mechanisms on alternations of 6 minutes, 8 seconds to 4 minutes, 20 seconds or 2-1/3 hours to 1-1/2 hours on and off, flowered a few days earlier due to the greater dosage of light (about 7 hours per night in each case) but were intermediate in height between those not lighted at all and those on thermostatic regulation of lighting. High temperature (75° at night) can be substituted for light in hastening flowering but produces very short plants.

There was no difference in the number of flowers per plant in any of

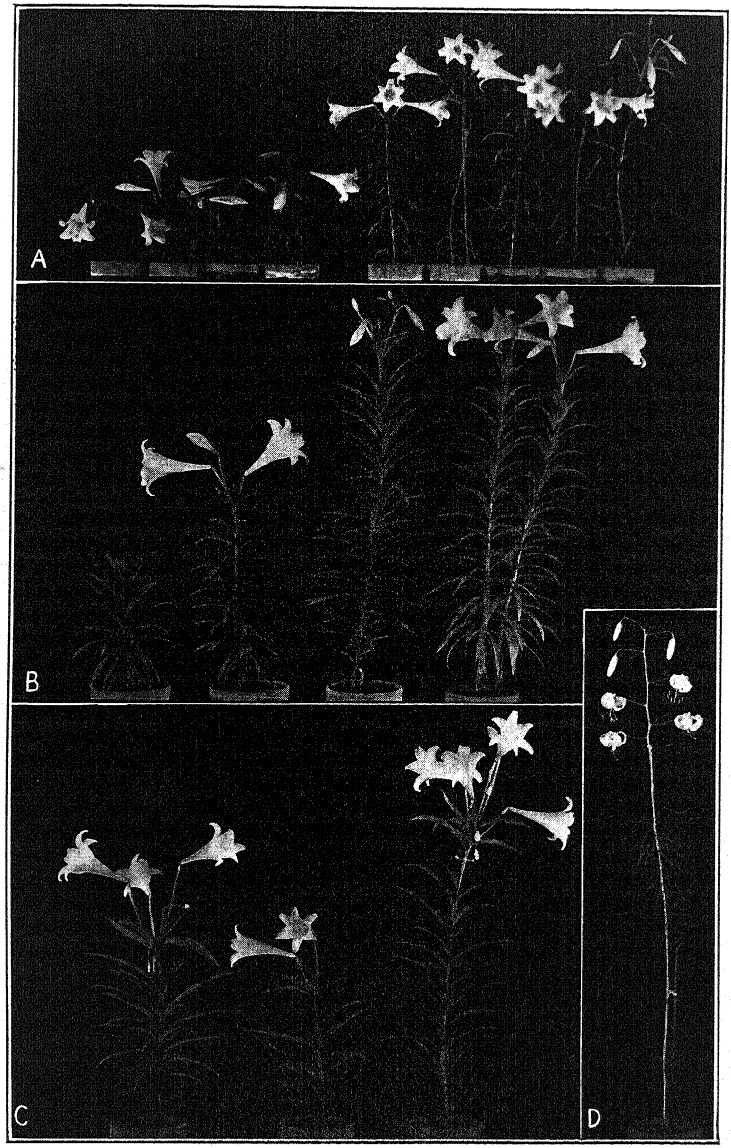

FIGURE 4. Lily. (A) *L. harrisii*, planted September 17, photographed December 24, four at left grown at 55°–60° F. control greenhouse, five at right same temperature but given intermittent light each night. (B) Giganteum, planted January 3, photographed April 25, left to right: (1) temperature 55°–60° F. control greenhouse; (2) temperature 75°, no extra light; (3) and (4) temperature 55°–60° F. intermittent light, (3) general thermostat control circuit, and (4) short period intermittency (clock mechanism). (C) Erabu, planted January 3, photographed April 25, left to right: (1) control greenhouse 55°–60° F.; (2) control at 75° F.; (3) intermittent light, general thermostat circuit 55°–60° F. (D) *L. tenuifolium*, planted January 3, photographed March 3, intermittent light, short period (clock mechanism) 55°–60° F.

the conditions, except that at high temperature under continuous lighting for 6 hours each night a few buds aborted and dropped off. Out of 46 plants lighted in the cool greenhouse 10 plants produced 1 flower per plant, 15 produced 2 flowers, 15 produced 3 flowers, 4 produced 4 flowers, and 2 produced 5 flowers. The number of flowers produced apparently depended upon the size of the bulbs at the start and not on subsequent lighting conditions.

A second crop of bulbs was planted on January 3 to determine whether another crop could be grown in time for Easter. Erabu and Giganteum were used as the two *longiflorum* types, and *L. tenuifolium* as another promising greenhouse variety for these tests. Some of the bulbs were placed at once in a high night temperature greenhouse (75°) and others were placed under the bench in the lighted house at a lower night temperature (55°–60°) until the plants appeared above the soil. They were then placed upon the benches in the various greenhouses. Several pots of bulbs were again placed in the cold dark room at 50° until February 8 or March 8 when they were returned to the different greenhouses. The lower greenhouse temperature (55°–60°) was found to be better for the germination of the bulbs, resulting in a more uniform stand. This crop of bulbs had probably little or no cold storage treatment before shipment to the Institute in contrast with the first crop. This may account for their slow germination at higher temperatures. Many of the *L. tenuifolium* plants developed a wilt disease at the higher temperature and only one out of 16 plants produced flowers in the 75° house, while only two out of 16 failed to produce flowers at 55°–60°. The addition of 6 hours of light each night in this house did not help these plants. On the other hand both Erabu and Giganteum developed rapidly at this high temperature after they had emerged from the soil but several failed to germinate at once. All bulbs germinated at the lower temperature of 55°–60°. Additional light at the higher temperature accelerated growth and flowering of both varieties and increased the height but none of these plants were as large as those grown with intermittent light at the lower temperature. *Tenuifolium* (shown in Fig. 4 D) flowered first on March 4 under intermittent lighting (clock mechanism, 6 minutes, 8 seconds on, alternated with 4 minutes, 20 seconds off for 7 hours nightly), 60 days after planting. Plants came into flower under intermittent lighting operated by the thermostat in this same greenhouse (55°–60°) exactly one week later where less than one-half of the total hours of burning were used. Flowers first opened in the control house without additional light on March 22. Erabu first flowered at the high temperature under 6 hours per night of light on March 14 and Giganteum on April 1. Plants from both varieties were photographed April 25 and are shown in Figure 4 B and C. The dates of flowering were approximately the same in the low temperature house under intermittent lighting (clock mechanism, 6 minutes, 8 seconds on, alternated with 4 minutes, 20 seconds off) and about one week later in the cool greenhouses on thermostat lighting. Plants given the cold dark room treatment of one month or two months were delayed as to flowering approximately one month and two months and the plants were shorter at full flowering than controls similarly treated except without cold storage. *L. tenuifolium* developed the most rapidly of the three types grown in the second crop. Figure 4 D shows a plant grown in exactly two months from the time the bulb was planted. Erabu was second and Giganteum was third. The condition of some of the plants on April 25 is shown in Figure 4 B and C.

Oriental poppy. Approximately 12 out of 18 potted plants of this species flowered under intermittent lighting (thermostat control) during the season of 1936–1937 (night temperature 60°–65°) when brought in from October to December from an unheated greenhouse. Some flowered when brought in on March 30. Other plants brought in from October to December were kept in a 65° greenhouse (night temperature) without lights. These were placed in the lighted greenhouse on February 11. They failed to flower; apparently holding the plant on a short day at 65° for a time produces dormancy of flower buds. This plant needs further study as to its exact temperature and light requirements. It is evident that with proper treatment it can be brought into flower at any time during the late fall or winter.

Strawberry. Plants of the Dorsett variety were grown under various lighting conditions at night temperatures of 55°–60°, 60°–65°, and 65°–70° F. The plants were found to fruit best at the lowest of these three temperatures. The past season's tests were conducted mainly at 55°–60°. The only departure from this was a brief test at 75° night temperature after fruit was set, which resulted in poor berry development either with or without light. Some of the plants in the past season's tests were kept in a cool dark room (50° F.) each night from June 22 to September 15 and were kept in a greenhouse each day. There was very little growth during this time. At the end of the cold treatment they were placed under intermittent light (6 minutes, 8 seconds on, alternated with 4 minutes, 20 seconds off, clock mechanism regulation) and given a total of about 7 hours of light each night. These plants grew and flowered rapidly and developed both runners and ripe berries by December 15. A photograph of one of these plants is included as Figure 5 A, plant 2. Plant 3 in this same figure was kept continuously in a high temperature greenhouse all summer and was then placed under the same temperature and lighting conditions as plant 2. It grew very little and developed no runners but flowered well and produced many ripe fruits. The yield in this crop of berries appeared to be better than that of the other plants which had the low temperature treatment, although the yield was good in both cases.

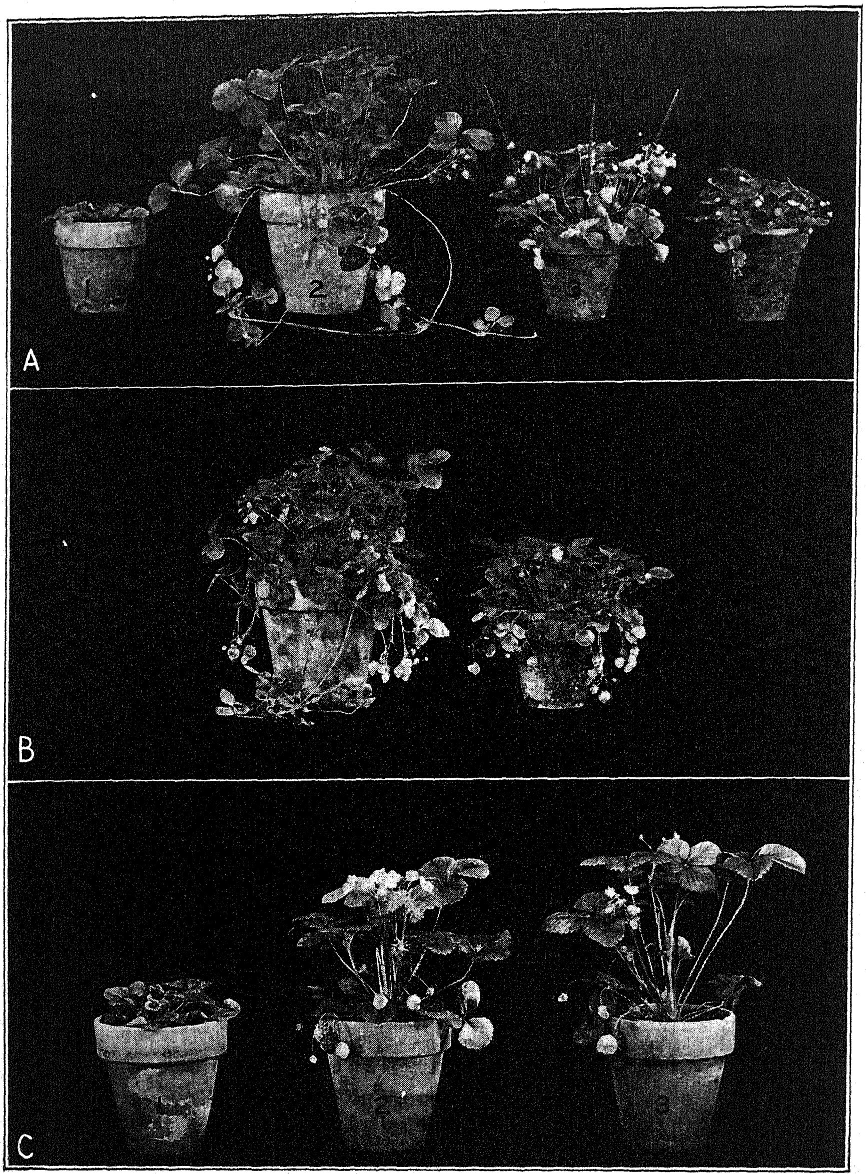

FIGURE 5. Dorsett strawberry, (A) photographed December 15, left to right: (1) brought in from outdoor cold frame December 10; (2) 50° F. cold room each night and greenhouse each day from June 21 to September 15, then intermittent light, short period (clock mechanism), 55°–60° F.; (3) same as (2) except given no cold treatment at night; (4) control greenhouse (same temperature, no extra light). (B) Same as (A), plants (2) and (3), photographed April 9, showing second crop of fruits. (C) Young Dorsett plants, January 13, 1936; left, control greenhouse; center, greenhouse with intermittent light (general thermostat circuit); right, insulated greenhouse.

Plant 1 in this figure was brought in from the cold frames five days before and plant 4 was from the control lot without light. The lighting was continued throughout the winter and the same plants were photographed again on April 9 showing the second crop of berries (Fig. 5 B). By June 21 the plants which had had the low temperature treatments had a third small crop of ripe berries, while those which had no cold treatment failed to produce again but were producing many runners as the temperature increased. It is therefore possible to produce rapid foliar development with runner production and fruit (cold treatment followed by higher temperature), or fruit only with very little foliar development and no runner production (no cold treatment). That is, foliar development is not always associated with flowering and fruiting in this plant. The tendency to become "ever-bearing" seems to be associated with low temperatures and long days of high intensity. Darrow (6) has studied light and temperature requirements of a number of varieties of strawberries including Dorsett and observed a decreased runner production and increased flowering when plants were grown at 60° as compared with 70° F. on 16 or 14 hours of illumination. Lower temperatures (55° F.) tended to produce dormancy in these plants. In the present series of tests it was observed that plants brought in from cold frames to the 55°–60° greenhouse on December 22 were slow to produce flowers and yield of fruits was poor. These plants had produced no runners by June 21. The plants receiving 7 hours of intermittent light each night (clock mechanism 6 minutes, 8 seconds on, alternated with 4 minutes, 20 seconds off) grew faster and yielded better than those receiving an average of 3.4 hours or less (thermostat mechanism). Preliminary tests indicated that the 85–watt capillary mercury lamp applied intermittently for 7 hours each night was sufficient to produce good flower and fruit development (clock mechanism 2-1/3 hours on, and 1-1/2 hours off). Further work needs to be done with more plants to determine whether such yields would justify the cost of lighting for commercial production for a December market. Plants grown without light at the low night temperature grew very slowly but were flowering and setting a few fruits on December 15 as shown in Figure 5 A, plant 4. A plant brought in from the cold frames one week before is shown as plant 1 of this same figure. Young plants rooted in July and August are shown in Figure 5 C. Plant 1 was grown in a greenhouse at 55°–60° without additional light, plant 2 received approximately 7 hours per night of intermittent light, and plant 3 was grown in the insulated greenhouse receiving less than one-half this amount of light (thermostatic control). Fruits of strawberries grown in the insulated greenhouse the year before at 60°–65° F. night temperature were very susceptible to molds or other fungi due to the high humidity maintained in this house. At the lower temperature 55°–60° F. the fruits were not attacked. The flavor of strawberries grown in this way seemed to be fully equal to that of those grown outside during the spring and summer months.

Sweet peas. These plants were grown in 2-gallon glazed earthenware jars at a night temperature of 60°–65° during the winter of 1936–1937. The plants came into flower first on January 11 in the lighted houses and on January 26 in the control house without light. The average yield of cut flowers per pot to March 31 was 77 for the controls, 120 for the lighted greenhouse, and 30 for the insulated house. The poor yield in the insulated house was due to the loss of many plants from wilt due to the high humidity. These plants were not grown in any of the houses during the last year at the lower temperature, 55°–60° F. It is not known whether the plants would be more resistant to the attacks of fungi in the insulated house at a lower temperature as was the case with strawberry. The plants reach the flowering stage earlier with intermittent lighting and the growth and flowering is also maintained at a higher level than control plants grown without light. In addition, the stems of the cut flowers are longer and more desirable commercially.

CARBON DIOXIDE SUPPLY

The importance of supplying additional carbon dioxide to the insulated greenhouse has already been discussed. Three methods were used to supply this gas in the tests reported herewith. All were found satisfactory but the relative advantages of each should be briefly considered. The first method used has already been described (4). A lump of solid carbon dioxide with an average weight of 40 pounds was sealed into a double walled, well insulated, sheet metal box. A small metal tube led out from the interior of the box and extended along the growing bench at a height of 3 or 4 inches above the soil. The upper part of this tube was perforated at 6-inch intervals and served to conduct the gas to the growing plants. Another method used to supply the gas was to attach a steel cylinder of carbon dioxide to this pipe and allow the gas to bubble slowly but continuously through a wash bottle to the delivery pipe. The third method of supplying this gas was the use of chickens which were chosen because of their high metabolic rate. The CO_2 output of a single 5-pound bird was determined and found to be approximately 540 cubic centimeters of CO_2 per hour while at rest. First ten and later only three birds were placed in a long cage extending the entire length of the house along the north wall as shown in Figure 6 A. The approximate volume of the insulated greenhouse is 1368 cubic feet and it was calculated that approximately 11.6 liters of CO_2 would be required per hour to supply the normal amount in air, assuming a complete air change every hour. An average of six analyses of the greenhouse air using the Lundegårdh apparatus (12, p. 9–10) showed a concentration of 0.051 per cent CO_2 in the morning after the greenhouse had been closed throughout the night and with three birds in the cage. No doubt some of this gas arose from the fermentation of the litter in the cages. While the use of three fowls as a source of carbon dioxide was entirely adequate for the growing plants it was found that ammonia was given off from the droppings at the operating temperature of 65°–70° F. in sufficient volume to burn the leaves of the plants. *Pelargonium* and buckwheat plants injured in this way are shown in Figure 6 D and C, respectively. In later work the

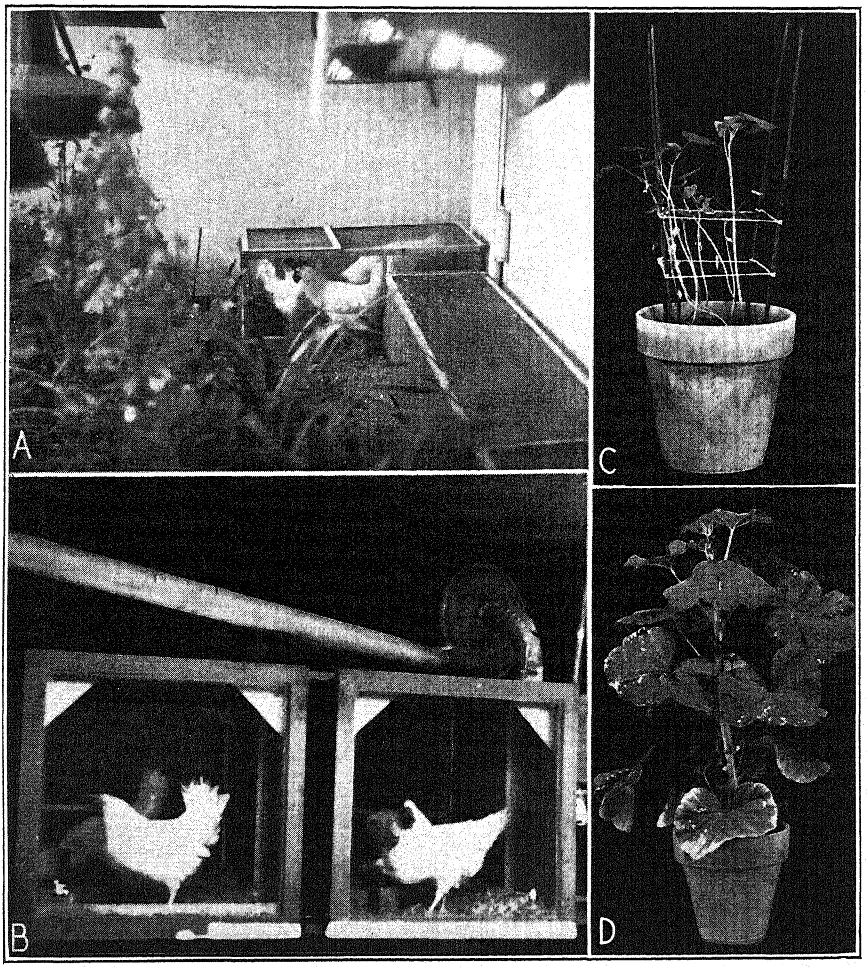

FIGURE 6. (A) Fowl cages inside the insulated greenhouse. (B) Outside chambers for fowls, showing blower arrangement for pumping air through water scrubber located inside greenhouse. (C and D) Ammonia burns on buckwheat and geranium plants, arising from droppings of birds.

birds were placed in a sheet metal chamber outside of the greenhouse and air from this chamber was pumped through a screen and a water filter of the bubbler type by means of a small fan and motor. This removed both the great quantities of dust or lint which is being constantly given off by fowl and the ammonia gas. A photograph of the cages and blower arrangement is shown as Figure 6 B. It is believed that this latter method of supplying CO_2 may be useful to those in rural sections who carry on poultry production along with other farm operations and wish to use an insulated greenhouse for plant production during the winter months.

DISCUSSION

From the data already presented it is evident that the ordinary type of greenhouse with intermittent lighting produces both greater dry weight and better flowering on many plants than the greenhouse without lights. The insulated greenhouse is also better in this respect during the winter months even without additional carbon dioxide, but not equal to the ordinary greenhouse with such lighting unless this gas is supplied. The reasons for this are quite evident. The ordinary greenhouse is designed with overlapping sheets of glass which admit of a rapid air exchange with the external atmosphere and therefore a frequent renewal of the gas at the same concentration as in air. This type of construction produces at the same time a greater heating load in order to maintain a given temperature inside the greenhouse and results in increased fuel costs. This rapid air exchange also makes it impractical to supply additional concentrations of carbon dioxide unless the gas is available at little or no cost. In a previous study (1) it was found that approximately 300 pounds per 24-hour day of this gas were required to maintain a concentration ten times the normal in a lighted basement room and one lighted greenhouse (19 by 25 feet). This would amount to approximately 150 pounds per day of the gas for the greenhouse alone if the above concentration were maintained continuously. Since the insulated greenhouse is of sheet metal it is of almost airtight construction when the windows and door are closed. This results in an extremely low rate of air exchange, low heat load to maintain a given temperature, and a high humidity. At the same time a small amount of additional carbon dioxide serves to increase the concentration of this gas over that found in normal air and it becomes a practical necessity to supply the gas in the insulated greenhouse. These factors should be carefully considered before deciding upon either type of construction.

Much has been written on the periods of alternation of light and darkness in intermittent lighting and the relative efficiency of photosynthesis and therefore in dry weight production. Garner and Allard (9) found a minimum for several plants at alternations of one minute on and off. Increases to 30 minutes or 1 hour or decreases to 5 seconds produced much better growth and dry weight production on several types of long and short day plants. Cosmos and delphinium reached a minimum at 1 minute and were best at either 12-hour or 5-second alternations of light and darkness. Gregory and Pearse (10) found a minimum opening of the stomata at equilibrium on alternations of 45 seconds. Emerson and Arnold (8) found an increase in photosynthetic yield of 300 to 400 per cent by using 50 flashes per second and making the light flash period much shorter. They found that the minimum required dark period was about 0.03 to 0.4 second depending upon temperature, as compared with a light period of 0.003 second or less. Finally McAlister (13) has developed a very rapid and accurate method for measuring carbon dioxide as it is used in photosynthesis. He found that after a wheat plant has been exposed to light a few hours, then allowed to remain in darkness for 10 minutes, the full photosynthetic rate is again established in approximately 2 minutes after the plant is lighted. Following a dark period of 10 hours, 12 to 15 minutes' exposure to light is required before the full rate is reached. He stated also that a minimum of photosynthesis probably falls between light exposures of 1- and 5-minute alternations. The usual increase in efficiency for periods shorter than 15 seconds (at 1/60 second on and off) was found and these values approached a 100 per cent increase over continuous light. When a plant was lighted in periods of 5 seconds on, alternating with 5 seconds off, the value 8.8 for photosynthesis showed only a slight improvement over plants lighted continuously for twice the time, with a photosynthetic value of 16.2.

In the data submitted in the present paper no advantage in dry weight production was found when 5-second alternations were compared with continuous light using all artificial light. Also, there was little difference when alternations of 6 minutes, 8 seconds on, and 4 minutes, 24 seconds off were compared with 2-1/3 hours on, and 1-1/2 hours off, during each night while each set received March sunlight during the day. The total illumination was approximately the same in each case. It may be that a shorter flash than that used in the first case will produce greater efficiency. This remains to be tried. It may also be that the 2-1/3-hour lighting interval will show a greater efficiency than the 6-minute, 8-second interval if tried in December or January when the number of hours of sunlight is less. The present data indicate, however, that with buckwheat, a plant not affected by daylength as to flowering, there can be wide differences in the periods of alternations of light and darkness with little difference in the dry weight produced, providing the total illumination received is the same. The fact that such good growth and flowering of many species have been produced when the lighting period was regulated by thermostats with variable frequency also strongly indicates that in practical greenhouse production the lighting frequency is not so important as control of the heat output.

On the other hand two lamps regulated by the soil heating thermostat suspended beneath one of them appeared slightly less effective than the other general lighting system regulated by the thermostat in the heat insulated greenhouse. This may have been due to the shorter burning time in the first case which was usually about 4 to 6 minutes as compared with 10 or 12 minutes for the general lighting system. Further tests are needed to determine the relative efficiency of short lighting periods followed by longer dark periods. While those plants under lamps regulated by the clock mechanisms developed faster than those regulated by the thermostat, the total illumination period each night in the former case was over twice as long (7 hours as compared with an average of 3.4 hours for the general lighting system). The difference in time required to reach the flowering stage in both lily and hyacinth was usually less than two weeks so that the use of twice as much light to produce flowering two weeks earlier on these plants was not justified. Also, the plants were usually better developed when grown at the slower rate with the lamps under thermostatic control. This method has so far proved especially satisfactory for lily, hyacinth, gardenia, gladiolus, calceolaria, begonia, snapdragon, stocks, and sweet peas. Strawberry responded better when grown under the lamps operated by the clock mechanisms which gave a total of 7 hours of artificial light each night. Apparently the small amount of light given by thermostatic regulation in October and November is insufficient for full development of these plants. Preliminary tests indicated that the light from the small 85-watt capillary mercury lamp when applied intermittently (2-1/3 hours on and 1-1/2 hours off) for 7 hours each night was sufficient to force good flower and berry development.

Many common greenhouse plants require a low night temperature for best growth and flowering. At the same time many also require high light intensity and long days as the most effective treatment for forcing flowers. This combination can best be supplied at present by using higher wattage lamps (500-watt) burning intermittently in comparatively short periods with the heat output accurately controlled by means of a thermostat. This system of heat control largely counteracts the poor energy distribution in the output from our most practical light source—the tungsten filament lamp.

SUMMARY

1. The growth and flowering of many kinds of plants have been studied in two types of greenhouses equipped for supplemental light at night. In one type, tungsten lamps furnished the sole source of both heat and light and in the other a part of the heating load was carried by a thermostatically controlled steam heating system. The greenhouse heated only by lamps was well insulated so as to reduce heat losses to a minimum. The same amount of light was applied to plants in each house, both circuits

being operated by a single thermostat located in the heat insulated house. An average of 3.4 hours of light per 24-hour day was supplied during December, January, and February.

2. Dry weight production and flowering were greatly increased in both lighted houses as compared with controls without additional light, but dry weight production was less in the heat insulated house than in the ordinary greenhouse with the same amount of light. This was found to be due to restricted carbon dioxide supply as a result of low rate of air exchange in the insulated house.

3. Three sources of carbon dioxide were used for increasing the concentration of this gas in greenhouse air: lumps of solid carbon dioxide, steel cylinders of liquid carbon dioxide, and the carbon dioxide respired by fowls. When the last source was used it was found necessary to place the fowls in an outside chamber and to filter the respired air through water to remove ammonia which arose from fermentation of the droppings.

4. Hyacinth bulbs were brought into flower in two to four weeks' exposure to light after a preliminary treatment of approximately three months in a dark room at 50° F. Higher temperatures during the light exposure increased the rate of development but decreased the quality and life span of the flowers.

5. The first crop of *longiflorum* lilies was grown for Christmas from bulbs planted September 17. The second crop planted January 3 was in flower the latter part of March in time for Easter. It was found that higher temperatures greatly shortened the time to flower production but greatly decreased the height of plants. The best lilies were produced in the shortest time at a night temperature of 55°–60° F. under the system of intermittent lighting. *L. tenuifolium* was the first to flower, then Erabu, and later Giganteum of those tested at the same time. Low temperature treatment greatly retarded development of the lily.

6. Both the garden varieties and large flowering types of gladiolus were brought to flower in late January and February from bulbs planted October 5 when grown at low temperature with intermittent light for an average of 3.4 hours each night. The flowers were cross pollinated and produced large quantities of viable seed.

7. Assuming a cost for current of two cents per kilowatt, the total cost per flower spike for gladiolus is approximately five cents if this method of lighting is continued until the last of January. This cost would also include the total heating cost in the insulated greenhouse and a large part of the heating cost in the ordinary greenhouse lighted each night.

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SIMMONDSIA CALIFORNICA NUTTALL IS DIOECIOUS

Frederick Gibson[1]

When Link (5) described his *Buxus chinensis* without certainty of habitat and guessed that it was from China, he started a series of errors regarding this plant. Aside from the error in name, he failed to describe it as being dioecious and all other authors since, with the exception of Engler and Prantl (2), have likewise overlooked this habit of inflorescence. Most of the descriptions would lead one to infer that both staminate and pistillate flowers are borne on the same plant.

The following statements regarding the inflorescence have been made: (5, pt. 2, p. 386), "floribus axillaribus solitariis"; (7), "Male flowers axillary, clustered, herbaceous; those of the female flower generally solitary"; (4), "*Simmondsia pabulosa.*" "Sterile flowers numerous in axillary dichotomous panicles, shorter than the leaves, seldom sessile and solitary, or in small glomerules"; (10, p. 202), no reference to the flowers, but the plant is placed with the Euphorbiaceae; (12, p. 67), "staminate flowers clustered upon short axillary peduncles or in short terminal compound racemes, the pistillate axillary and solitary"; (2, p. 135), "Diöcisch." The explanation of the illustrations clearly states that the flowering twigs are from male and female plants; (8, p. 141), "*Simmondsia chinensis* (*S. californica*)." "Bl. dioecisch"; (9, p. 654), "flowers dioecious, the staminate in sessile or pedunculate clusters, the pistillate solitary, on recurved pedicels"; (9, p. 1671), *S. chinensis* (Link) Schneider is adopted as the valid name; (1, p. 217), the same wording as (12) above; (3, p. 607), "staminate flower 1-1/2 to 2 lines long, its sepals broadly oblong or subspatulate; pistillate flower much larger than the staminate"; (6, p. 291), generic description; "Flowers on short axillary peduncles; the staminate clustered; pistillate solitary"; (11, p. B148), "The male (staminate) and female (pistillate) flowers are borne separately on the same plant; the former in clusters and the latter solitary."

Attention is here called to the fact that the plants are strictly dioecious. We have counted the sexes as they appear in nature and found them to be balanced. One might be easily confused about the flowering habit, because the individual plants may grow so close together and the branches intermix in such way as to give the appearance of being a single shrub.

This dioecious character has an economic relation to the future possibility of growing jojoba (*Simmondsia californica* Nuttall) plants for commercial use of the seed oil, known as jojoba oil. Those who contemplate such plantations should know that if they set out seedlings, only half will be seed-bearing.

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EXPERIMENTS ON GREENHOUSE FUMIGATION WITH β, β'-DICHLOROETHYL ETHER[1,2]

FRANK WILCOXON AND ALBERT HARTZELL

In 1937 Campbell and Stone (1) published a note on the successful use of dichloroethyl ether as a soil fumigant in the control of wireworms, *Limonius* (*Pheletes*) *californicus* Mann. In the same year Stone and Elmore (2) described the control of sod webworms, *Crambus* sp., on lawns, by sprinkling infested areas with an aqueous solution of this compound. No experiments have yet been reported on the use of dichloroethyl ether as a greenhouse fumigant.

The present paper presents the results of a number of greenhouse fumigations with dichloroethyl ether, in which the toxicity to insects, the plant tolerance, and the effect of various methods of volatilization were studied.

PRELIMINARY EXPERIMENTS

In the first experiments a fumigation box was used, with a capacity of 35 cu. ft. The material was volatilized with the aid of a small electric fan, and both aqueous solutions and the pure substance were tested. Encouraging results were obtained by both methods, and subsequent fumigations were carried out in a larger greenhouse compartment with a capacity of 850 cu. ft., and eventually in the greenhouse itself.

FUMIGATIONS IN GREENHOUSE COMPARTMENT

The compartment was situated between two larger sections and its height and width were the same as that of the adjacent greenhouse sections and differed only in being one-seventh the length of the latter.

Three different methods of volatilization were used, as follows: (a) volatilization from aqueous solution using the fumigation machine described in a previous publication (3); (b) volatilization from aqueous solution in shallow pans using an electric fan; (c) volatilization of the pure material with an electric fan. In the case of methods (b) and (c) in order to control the rate of evaporation porous carbon plates[3] (Grade 4036), with a total area of approximately 52 sq. inches, were suspended vertically over shallow pans containing the solution or the pure material, arranged in such a manner that the bottom edge dipped into the liquid (Fig. 1 A).

In this manner the effective evaporation area could be varied, since

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[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 180.

[2] The materials for this investigation were furnished by the Carbide & Carbon Chemicals Corporation, New York, N. Y.

[3] The plates were furnished by the National Carbon Co., New York City.

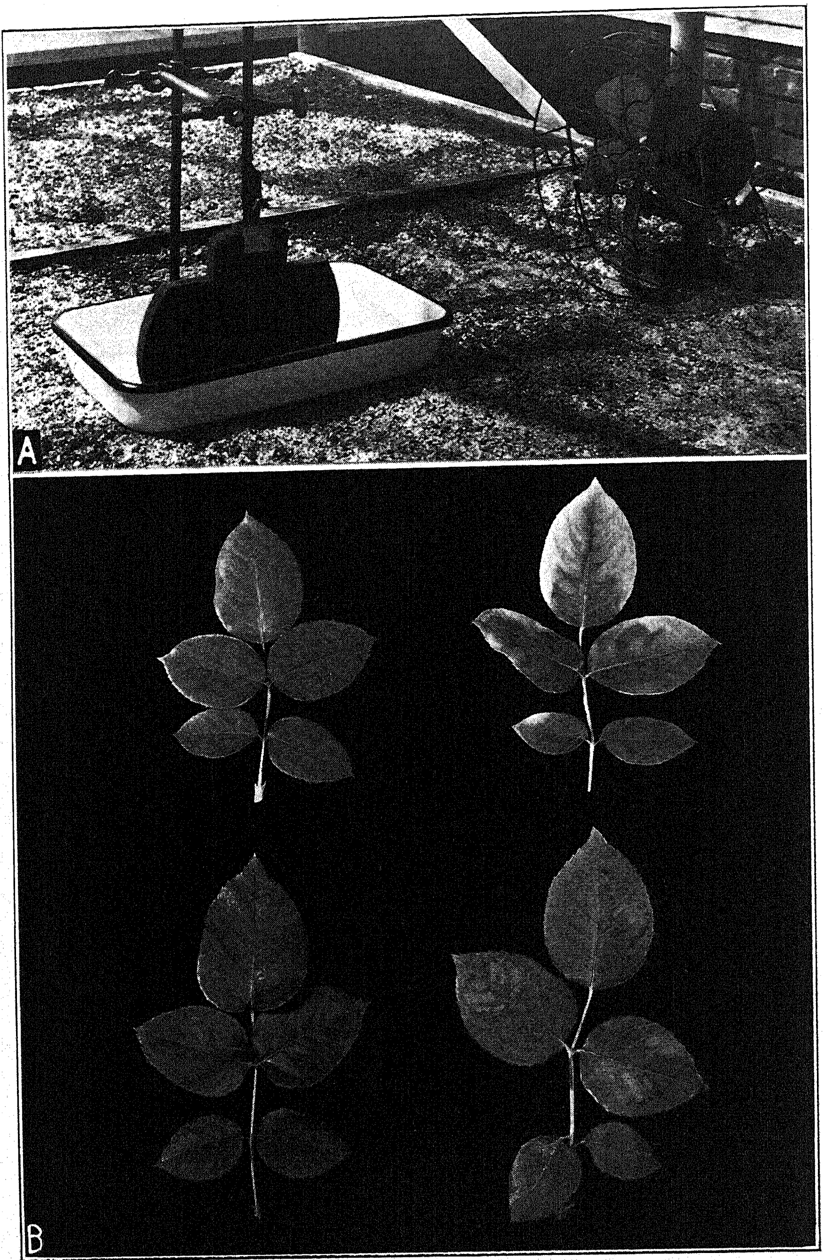

FIGURE 1. Dichloroethyl ether fumigation. (A) Apparatus consisting of porous plate, shallow pan, and electric fan for volatilizing dichloroethyl ether in greenhouse. (B) Dichloroethyl ether injury to roses. Above, variety Hollywood; below, variety Briarcliff. Check on left, in each case.

the capillary action of the plates caused them to become wet throughout with the liquid. Successful results were obtained by methods (a) and (c), but method (b) was unsatisfactory due to the low toxicity to insects. Method (a) involves the use of the fumigation machine, which is necessarily cumbersome and expensive, and therefore in later work emphasis was placed on method (c) which required a much smaller volume of liquid, less space, and less expense.

A typical result obtained by method (a) follows: Using a saturated solution of dichloroethyl ether, 236 cc. to 7.5 gal. of water in the fumigation machine, with a 15-hour fumigation period, a 99 per cent kill was obtained of red spider mite (*Tetranychus telarius* L.), 99.5 per cent kill of *Aphis rumicis* L., 100 per cent kill of gladiolus thrips (*Taeniothrips simplex* Morison), and 100 per cent kill of adult white fly (*Trialeurodes vaporarium* Westw.). The temperature range was 68° to 88° F.

FUMIGATIONS IN GREENHOUSE

The following 44 species of plants were tested: *Acidanthera bicolor* Hochst., African daisy (*Dimorphotheca* sp.), African violet (*Saintpaulia ionantha* Wendl.), *Aloe arborescens* Mill., *Begonia* sp., *Beloperone* sp., buckwheat (*Fagopyrum esculentum* Moench.), carnation (*Dianthus* sp.), *Chrysanthemum* sp., castor bean (*Ricinus communis* L.), *Coleus blumei* Benth., corn (*Zea mays* L.), cotton (*Gossypium* sp.), *Dahlia variabilis* Desf., dwarf and climbing nasturtium (*Tropaeolum majus* L.), Easter lily (*Lilium longiflorum* Thunb.), English ivy (*Hedera helix* L.), fig (*Ficus* sp.), *Fuchsia hybrida* Voss, *Gardenia jasminoides* Ellis, geranium (*Pelargonium hortorum* Bailey), *Gladiolus* sp., gloxinia (*Sinningia speciosa* Benth. & Hook.), *Hibiscus* sp., holly (*Ilex opaca* Ait.), *Kalanchoe* sp., morning glory (*Ipomoea purpurea* [L.] Roth), *Nigella* sp., oleander (*Nerium oleander* L.), peach (*Prunus persica* [L.] Stokes), *Peperomia* sp., pepper (*Capsicum frutescens* L.), *Petunia* sp., *Phacelia* sp., pineapple (*Ananas sativus* Schult.), poinsettia (*Euphorbia pulcherrima* Willd.), *Salpiglossis sinuata* Ruiz & Pav., *Sansevieria* sp., sweet pea (*Lathyrus odoratus* L.), tobacco (*Nicotiana tabacum* L. var. Turkish), tomato (*Lycopersicon esculentum* Mill. var. Bonny Best), rose (*Rosa* [hybrid tea] vars. Briarcliff, Hollywood), wild plum (*Prunus americana* Marsh.), *Zinnia* sp.

The plants were grown in the greenhouse. Four potted plants of each species were tested as a rule in each experiment. In general there was but one plant per 4-inch pot except where the growth habit allowed more. Buckwheat and nasturtium were grown four to ten plants per pot. Gladiolus was grown principally in flats, 12 plants to a flat. The roses were grown in the greenhouse bench. All the plants received the same greenhouse treatment and were watered about two hours before fumigation. At the end of the fumigation the plants were kept under observation for several weeks in the greenhouse. Some of the tolerant species received repeated fumigations.

The insects and mites tested included *Aphis rumicis* on nasturtium, red spider mite on rose, and gladiolus thrips on gladiolus. It was noted that the aphids with this fumigant had a tendency to leave the host plant so that in order to obtain a true mortality value, infested plants from which counts were made were enclosed in cheesecloth to prevent migration of the aphids.

METHOD (A)

Five fumigations have been run by this method in a greenhouse, the capacity of which was 6228 cu. ft. It was found that the relative rates of evaporation of dichloroethyl ether and water, in the fumigation machine, were not equal. A sample drawn at the end of a 15-hour fumigation period showed that 85 per cent of the dichloroethyl ether had been volatilized, while only 11.1 per cent of the water had evaporated. The experimental conditions and the results are shown in Tables I, II, and V, with the exception of those species of plants which showed no injury under any of the experimental conditions.

TABLE I

DEGREE OF INJURY* TO PLANTS BY DICHLOROETHYL ETHER AT DIFFERENT CONCENTRATIONS AND PERIODS OF FUMIGATION. TEMPERATURE 60° TO 102° F. RELATIVE HUMIDITY 64% TO 90%

Plants	315 cc./10 gal., 17 hr.	283 cc./9 gal., 16 hr.	283 cc./9 gal., 14 hr.**	267 cc./ 8-1/2 gal., 14 hr.	236 cc./ 7-1/2 gal., 14 hr.
Buckwheat	—	o	o	x	—
Nasturtium (dwarf)	—	—	x	o	o
Coleus	x	—	—	—	—
Carnation (red)	o	xxxx	xxxx	xxxx	—
Cotton	xxx	o	o	o	o
Gardenia	xxx	o	o	—	o
Geranium (red)	xxx	o	o	—	o
Peach	—	xxx	—	—	—
Petunia	o	xxx	o	xxx	o
Salpiglossis	o	xxx	o	—	—
Tomato var. Bonny Best	xxx	xxx	xxx	xxx	o
African violet	xxxx	—	o	o	o
Beloperone sp.	xxxx	—	—	—	—
Gloxinia	xxxx	—	—	—	o
Phacelia	xxxx	—	o	—	o
Rose var.					
Briarcliff	xxxx	xxxx	xxxx	xxxx	—
Hollywood	xxxx	—	xxxxx	xxxxx	—
Plum (wild)	xxxx	xxxx	—	xxxxx	—

* o = none, x = very slight, xx = slight, xxx = moderate, xxxx = severe, xxxxx = very severe.

** Method (c); other columns give results of method (a).

TABLE II

EFFECT OF TEMPERATURE* ON DICHLOROETHYL ETHER (AQUEOUS SOLUTIONS) INJURY.**
RELATIVE HUMIDITY 64% TO 90%

Plants	69° F.	79° F.	82° F.
Buckwheat	—	o	o
Nasturtium (dwarf)	o	x	o
Coleus	x	o	o
Carnation (red)	—	xxxx	xxxx
Cotton	xxx	o	o
Gardenia	xxx	o	o
Geranium	xxx	—	—
Peach	o	o	xxx
Petunia	o	o	xxx
Salpiglossis	—	o	xxxx
Tomato var. Bonny Best	xxx	xxx	xxx
Beloperone sp.	xxxx	—	—
Gloxinia	xxxx	o	—
Phacelia	xxxx	o	—
Rose var.			
Briarcliff	xxxx	xxxxx	xxxx
Hollywood	xxxx	xxxxx	—
Plum (wild)	xxxx	o	xxxx
African violet	xxxx	o	o

* Temperature headings are averages of the ranges for the period of fumigation.
** See footnote* for Table I.

TABLE III

DEGREE OF INJURY* TO PLANTS BY PURE DICHLOROETHYL ETHER AT DIFFERENT CONCENTRATIONS AND PERIODS OF FUMIGATION. TEMPERATURE 71° TO 96° F.
RELATIVE HUMIDITY 48% TO 95%

Plants	660 cc., 17-1/2 hr.	330 cc., 17 hr.	220 cc., 17 hr.	220 cc., 10 hr.	220 cc., 10 hr.**
Buckwheat	o	o	—	—	—
Coleus (red)	o	o	xxx	o	—
Coleus (yellow)	o	o	xxx	—	—
Gladiolus var. Minuet	o	—	—	—	—
Nasturtium (climbing)	o	xxx	xxx	xx	xx
Nasturtium (dwarf)	o	o	o	xx	xx
Geranium	—	—	xx	—	—
Poinsettia	xxx	xxx	o	o	—
Castor bean	xxxx	xxxx	xxxx	xxxx	—
Chrysanthemum	xxxx	xxx	xxx	—	—
Fig	—	xxxx	—	—	—
Holly	xxxx	—	o	—	—
Rose	xxxx	xxxx	xxx	xxxx	xx
Tomato var. Bonny Best	xxxx	xxx	xxx	xxx	xxx
Peach	xxxxx	xxxxx	xxxx	xxxx	o
Plum (wild)	xxxxx	xxxx	—	xxxxx	—
CUT BLOOMS					
Rose buds	xxxx	—	xxxx	—	—
Sweat pea (3 pink)	xxxx	—	—	—	—
Sweet pea (white)	o	—	—	—	—

* See footnote* for Table I.
** Two fans used and two shallow pans and porous plates placed at opposite ends of the greenhouse.

METHOD (C)

Five fumigations were made by this method in a greenhouse, the capacity of which was 5365 cu. ft., and the results appear in Tables III, IV, and V.

TABLE IV

EFFECT OF TEMPERATURE* ON DICHLOROETHYL ETHER (PURE) INJURY.**
RELATIVE HUMIDITY 48% TO 95%

Plants	78° F.	82° F.	87° F.
Coleus (red)	o	o	xxx
Coleus (yellow)	o	o	xxx
Nasturtium (climbing)	x	xxx	xxx
Nasturtium (dwarf)	x	xx	o
Geranium	—	—	xx
Poinsettia	xxx	xxx	o
Tomato var. Bonny Best	xxxx	xxx	xxx
Castor bean	xxxx	xxxx	xxx
Chrysanthemum	xxxx	xxxx	xxx
Holly	xxxx	x	o
Rose	xxxx	xxxx	xxx
Peach	xxxxx	xxxxx	xxxx
Plum (wild)	xxxxx	xxxx	—
Fig	—	xxxx	—
CUT BLOOMS			
Rose	xxxx	—	xxxx
Sweet pea	xxxx	—	—

* Temperature is an average of the range.
** See footnote* for Table I.

PLANTS NOT INJURED BY DICHLOROETHYL ETHER

The following plants were not injured by dichloroethyl ether:

Acidanthera bicolor
Aloe
Begonia (variegated)
Beloperone sp.
Carnation (white)
Chrysanthemum
Coleus (red)
Coleus (yellow)
Corn
Dahlia
Easter lily
English ivy
Fuchsia
Gladiolus var.
  Ave Maria
  Alice Tiplady
  Edith Robson
  Miss Greeley
Gladiolus var.
  Mrs. Frank Pendleton
  Mrs. F. C. Peters
  Señorita
  Salmon Star
Geranium (red)
Hibiscus
Kalanchoe
Morning glory
Nasturtium (climbing)
Nigella
Oleander
Peperomia
Pineapple
Poinsettia
Sansevieria
Tobacco var. Turkish
Zinnia

TABLE V

CONTROL OF GREENHOUSE PESTS WITH DICHLOROETHYL ETHER VAPOR

Exposure in hours	Temperature, ° F.		Relative humidity, %		Concentration		*Aphis rumicis*		Red spider mite		Gladiolus thrips	
	Max.	Min.	Max.	Min.	Dichloro-ethyl ether in cc.	Water in gallons	Number counted	% dead	Number counted	% dead	Number counted	% dead
Method (a)												
17	76	62	—	—	315	10	210	96.7	268	97.3	72	100
14	88	58	—	—	236	7-1/2	169	20.7*	127	39.3*	25	80
14	90	68	—	—	283	9	—	—	242	96.2	40	100
14	94	70	90	64	267	8 1/2	109	89.9	143	75.5	50	100
Method (b)												
17	76	60	—	—	283	9	115	82.6	121	54.5	30	96.6
16	102	62	—	—	283	9	53	94.5	45	62.2	50	100
Method (c)												
17-1/2	85	72	95	80	660	None	118	100	107	100	50	100
17	92	72	85	48	330	None	23**	95.6	125	100	50	100
17	96	79	91	69	220	None	116	85.0	106	96.2	—	—
10	90	74	83	76	220	None	101	95.0	150	100	—	—
11-1/2	87	71	81	79	220	None	62	87.0	104	100	—	—

* Unsatisfactory kill due to low temperature.

** The infested nasturtium plant was not enclosed in cheesecloth to prevent migration of aphids. Figure includes only those aphids that remained on the plant after fumigation.

PLANT TOLERANCE

There was little or no evidence of injury to the plants immediately after fumigation. The injured areas exhibited wilting within 24 hours and usually became necrotic in 48 hours. Leaves of old tomato plants especially showed a chlorotic condition. In the case of roses the symptoms are delayed as injury to the canes was not observed for a week or ten days after fumigation.

A brief description of injuries caused by dichloroethyl ether vapor in detail follows:

Carnation (*red*). The flowers were bleached and streaked with white.

Castor bean. The older foliage was severely burned. The younger leaves showed definite light brown necrotic areas.

Chrysanthemum. The older leaves were scorched.

Peach. The growing point was injured. The older leaves had definite necrotic areas. At low concentrations there was no injury.

Plum (*wild*). The leaves were severely scorched.

Rose var. Briarcliff. The petals showed bleached areas. The older leaves were scorched (Fig. 1 B). Repeated fumigations killed the shoots by girdling, and the plants grew out below the graft in some cases. There was a delay in full expression of symptoms.

Rose var. Hollywood. The older leaves were scorched (Fig. 1 B), and the lower leaves were chlorotic. The petals were bleached.

Tomato var. Bonny Best. Young plants were injured. In older plants there was excessive guttation and epinasty following fumigation. The lower leaves were chlorotic. Generally there was a delay in full expression of symptoms. At lower concentrations there was no injury.

The effect of temperature on plant injury is not apparent in Tables I and III. However, when the individual tests are examined separately it will be noted in Tables II and IV that in certain tests such as the ones with cotton, gardenia, and poinsettia, injuries were obtained at lower temperatures and not at higher temperatures, whereas with certain other tests such as the ones with coleus, peach, and petunia, injuries were obtained at the higher temperatures but not at the lower temperatures.

TOXICITY TO INSECTS

It was found that dichloroethyl ether fumigation would control *Aphis rumicis*, red spider mite, gladiolus thrips, and adult white fly. This fumigant seems to kill a wider range of greenhouse pests than naphthalene as the latter is not very effective in the control of aphids or white fly. It required a relatively long time for dichloroethyl ether to give a complete kill. For this reason, counts of living and dead insects were not made immediately after fumigation but 48 hours later.

TESTS WITH OTHER CHEMICALS

The following compounds tested were unsuccessful as fumigants because they caused severe injury to plants: triethylene glycol (pure), mesityl oxide (pure and aqueous solutions), ethylene chlorhydrin (40 per cent).

DISCUSSION

Of the three methods of volatilization used, method (a) involving the use of the fumigation machine appears to be the most satisfactory from the point of view of plant tolerance and successful control. Method (b) in which the aqueous solution was volatilized from shallow pans with the aid of electric fans did not give satisfactory kills even when the previously described porous plates were used. Method (c) which has the great advantage of reducing the space required to a negligible point, since an aqueous solution was not used, required careful adjustment of the amount of fumigant, and the length of fumigation period to obtain satisfactory results. Nevertheless this method is the one which appears to be of the most practical use.

It seems unlikely that this fumigant can be used with safety on roses. It has an advantage over naphthalene in that no permanent odor nor taste is imparted to fruits of cucumber and tomato as is the case with naphthalene. Red carnations should be fumigated when the plants are not in bloom to avoid bleaching of the flowers.

The fact that dichloroethyl ether has a wide range of toxicity to different species of insects not possessed by other fumigants is another point in its favor. Further work with this substance on a commercial scale under varied conditions will be needed in order to determine its ultimate value as a greenhouse fumigant.

SUMMARY

β, β'-dichloroethyl ether has been tested as a greenhouse fumigant. It has been shown to control *Aphis rumicis*, red spider, gladiolus thrips, and adult white fly. Plant tolerance has been tested on 44 species of plants. Among the more susceptible species were rose, peach, castor bean, and carnation. Three different methods of volatilization of the fumigant were studied, and the most promising from a practical standpoint was the use of the pure compound in shallow pans, with vertical porous plates dipping in the liquid to give increased evaporation area. An electric fan was used to maintain circulation of the greenhouse air.

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ON THE BEHAVIOR OF PLANT FIBERS DISPERSED IN CUPRAMMONIUM HYDROXIDE SOLUTION[1]

JACK COMPTON[2]

The current concepts regarding the nature of the colloidal solutions resulting from the dispersion of cellulosic materials by cuprammonium hydroxide solution, Schweizer's reagent, depend on the theories of plant fiber structure, namely, the continuous or macro molecular theory, and the discontinuous or micellar theory.

The continuous or macro molecular theory, developed by Staudinger on the basis of viscosity measurements, may be summed up in the following statement (25): "Accordingly cellulose and its derivatives are molecular colloids and on the length and form of their macro molecules depends the colloidal behavior of their solutions." The cellulose macro molecules in plant fibers are considered regularly lined up under the influence of covalent forces in a direction parallel to the fiber axis.

The discontinuous or micellar theory, postulated by Nägeli (21) and extended by Karrer and Widmer (17) in 1921, assumes that the micelles of the cellulose component are formed by the union, or bundling, of many molecules of the elementary substance. Hess and coworkers (12, 13, 14), in applying the law of mass action to the optical behavior of purified plant fibers dispersed in cuprammonium solution, obtained results that were best explained when the cellulose molecule was assumed to be $C_6H_{10}O_5$. To explain the high viscosity of plant fibers in cuprammonium solution on the basis of so small a unit, he assumed that the $C_6H_{10}O_5$ groups are associated to form a larger molecular aggregate. Later Hess (10, p. 301) concluded that plant fibers should be regarded as a physical aggregation of small units and Meyer and Mark (20) postulated that these units, or cellulose micelles, were formed from primary valence chains held together by "micellar forces." Recently, Lieser (18, p. 291), in summing up his results, writes: "It may be stated as most important that cellulose in viscose and ammoniacal copper oxide forms micellar solutions, in organic bases and inorganic acids, solutions with a not yet clearly definable character. It was further shown that all celluloses regenerated from solution are of micellar structure." The dimensions of the cellulose micelle in plant fibers have been estimated (9) to be approximately 50 Å in diameter and over 500 Å long.

The observations of Farr and Eckerson (6, 7) that cottonseed fibers

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are composed of microscopic, ellipsoidal, cellulose particles of uniform size ($1.1 \times 1.5\mu$), arranged with their long axes parallel to the fiber axis and held together by an intercrystalline "cementing" material, are of fundamental importance. Cellulose particles obtained from cottonseed fibers possess all the properties recognized as characteristic of purified plant fibers other than high viscosity in cuprammonium solution. The low viscosity of the separated particles is attributed by Farr (5) to the removal of the viscosity producing "cementing" material. Qualitative examination of cuprammonium dispersions of cottonseed fibers with the slit ultra microscope, or working carefully with the microscope, revealed the presence of the apparently unchanged cellulose particles. Upon coagulation, these dispersions yielded a product containing randomly distributed particles similar to the original aggregates. From a study of the X-ray diffraction patterns of disintegrated cottonseed fibers, Sisson (22) has concluded that a microscopic unit of cellulose particle dimensions ($1.1 \times 1.5\mu$) satisfies all the fundamental requirements of the Nägeli micelle with the exception of size.

At present there are many divergent views regarding the chemical and physical nature of the cellulose component of plant fibers after dispersion in reagents such as cuprammonium solution. The purpose of the present investigation is: first, to study the entire cuprammonium viscosity range of cottonseed fibers to determine if, at any time, a relation exists between the viscosity and optical rotation of the cuprammonium dispersions; second, to determine in general the contribution of each component of native cottonseed fibers, i.e., crystalline cellulose particles and the intercrystalline phase, to the phenomena of viscosity and optical activity of cuprammonium dispersions; third, to study quantitatively the effect of varying the concentration of differently treated plant fibers dispersed in cuprammonium solution on the microscopic particle count, using the slit ultra microscope; fourth, to study the mechanism of the dispersion phenomenon of crystalline cellulose particles by cuprammonium solution.

EXPERIMENTAL

APPARATUS

The equipment of the Leitz slit ultra microscope included a micro cell and an eyepiece micrometer ruled in 1.0 mm. squares. In all counts herein reported a 6 L objective and a 10× eyepiece were used.

The optical rotations were determined in a Schmidt and Haensch polariscope with an 85-watt capillary mercury arc lamp as the light source. A combination of monochromatic glass filters (No. 7) recommended by the Corning Glass Works was used to transmit only the 0.4358μ blue mercury line.

Viscometers of the type recommended by Clibbens and Geake (2), slightly modified by the addition of ground glass stoppers in place of the rubber stoppers previously specified, were used in determining the viscosities of the variously treated plant fibers dispersed in cuprammonium solution.

PREPARATION OF THE CUPRAMMONIUM SOLUTION

The cuprammonium solution prepared by the method of Clibbens and Geake (2), containing 15 g. of copper, 240 g. of ammonia, and 1 g. of sucrose per liter was satisfactory for determining the viscosity, optical activity, and particle counts of the various plant fiber dispersions. It was possible to carry out all three determinations on one dispersion when necessary.

ANALYSIS OF CUPRAMMONIUM SOLUTION

The cuprammonium solution was analyzed essentially in the manner described by Dorée (4, p. 55).

The copper content was determined iodometrically in the following manner: a sample of the cuprammonium solution (1 cc.) was pipetted into a 50 cc. Erlenmeyer flask containing 5 cc. of distilled water. The ammonia was boiled off and the solution acidified with a few drops of concentrated sulphuric acid and boiled a few minutes longer. A small crystal of urea was then added to expel the last traces of nitrogen (HNO_2). The solution was cooled, made basic with ammonium hydroxide, and acidified by adding 3 cc. of glacial acetic acid. After again cooling to room temperature, 2 cc. of a 25 per cent iodate-free KI solution was added, and the liberated iodine immediately titrated with 0.02 N thiosulphate solution, using freshly prepared starch indicator.

The ammonia was estimated by adding 2 cc. of cuprammonium solution to 18 cc. of cold 2 N sulphuric acid and titrating back with normal alkali, using methyl red as indicator. This requires the subtraction of $0.536 \times (C)$ g. per liter of ammonia where (C) is the number of grams of copper per liter.

The nitrous acid content was found by determining the volume of cuprammonium solution necessary to decolorize 10 cc. of 0.1 N potassium permanganate in the presence of excess dilute sulphuric acid at 50° C.

THE EFFECT OF VARIOUS TREATMENTS OF COTTONSEED FIBERS ON THE VISCOSITY, OPTICAL ROTATION, AND PARTICLE COUNT AFTER DISPERSION IN CUPRAMMONIUM SOLUTION

Raw cottonseed fibers (*Gossypium hirsutum* L.) were extracted with alcohol-benzene (1:1) in a Soxhlet extractor for 24 hours, thoroughly washed with water, and air-dried at room temperature. This material was used in the following experiments:

A. *Cottonseed fibers treated with 0.01 N HCl solution at 75° C.* (16). Ten grams of cottonseed fibers were placed in one liter of 0.01 N HCl solution previously heated to 75° C. and after vigorous stirring and shaking, samples were removed at stated intervals of time (Table I). The sample was rendered immediately neutral after removal by plunging it into a large volume of ice-cold sodium bicarbonate solution. After agitating for ten minutes the sample was thoroughly washed with distilled water and air-dried at room temperature.

TABLE I

VISCOSITIES AND OPTICAL ROTATIONS* OF THE CUPRAMMONIUM DISPERSIONS OF COTTONSEED FIBERS AFTER TREATMENT WITH VARIOUS REAGENTS

Length of treatment	Cottonseed fibers treated with 0.01 N HCl at 75° C.		Cottonseed fibers treated with concentrated HCl (d. 1.18) at 25° C.		Cottonseed fibers treated with N/25 sodium hypochlorite at pH 9, 25° C.	
	$\eta_{c.p.}$	α	$\eta_{c.p.}$	α	$\eta_{c.p.}$	α
0 (min.)	46.0	−3.00°	46.0	−3.00°	46.0	−3.00°
5	—	—	3.4	3.12	—	—
10	35.3	3.01	2.7	3.09	—	—
15	—	—	—	—	22.5	3.06
30	28.6	3.06	2.2	3.06	18.4	—
1 (hr.)	25.3	3.01	2.0	3.02	16.3	3.02
3	17.1	3.02	1.9	3.04	12.1	3.03
4	14.4	3.01	—	—	—	—
4-1/2	—	—	—	—	10.4	3.03
8	10.3	3.01	—	—	—	—
8-1/2	—	—	—	—	7.9	3.06
22	—	—	1.9	3.02	5.0	3.01
32	4.5	3.04	—	—	—	—
Cottonseed fibers treated with excess H_2O_2 in presence of 4% NaOH at 100°; 10 hrs.					1.9	3.06

* All viscosities and optical rotations were determined with cottonseed fiber concentration of 0.5% (0.5 g. subs. in 100 cc. soln., moisture-free basis); the former at 25°, the latter in mercury light (435.8) at 32° in a 0.5 dm. tube. $\eta_{c.p.}$, cuprammonium solution 1.2.

B. *Cottonseed fibers treated with concentrated hydrochloric* (7) *acid* (*d. 1.18*) *at 25° C.* Cottonseed fibers (10 g.) were placed in concentrated hydrochloric acid (250 cc.) at 25° C. The removal and treatment of samples was effected in the manner described above (A). After 22 hours, slight pressure on the cover glass of a microscopic slide of this material destroyed the fiber structure completely. Many free cellulose particles could thus be disengaged from the fragments of fibers.

C. *Cottonseed fibers treated with N/25 sodium hypochlorite* (3) *solution of pH 9 at 25° C.* Ten grams of cottonseed fibers were treated with two liters of N/25 sodium hypochlorite solution containing 1.42 g. of available chlorine per liter, the solution being buffered at pH 9. Samples were removed at definite intervals of time (Table I), immediately washed with water, and given the standard acid wash. After again thoroughly washing in water the samples were air-dried at room temperature.

D. *Cottonseed fibers treated with excess hydrogen peroxide in the presence of 4 per cent sodium hydroxide solution* (16). Five grams of cottonseed fibers were placed in 200 cc. of 4 per cent sodium hydroxide solution and heated to boiling during the portion-wise addition of 3 per cent hydrogen peroxide solution over a period of 10 hours. The fiber structure could be completely disrupted by pressure at the end of this time, yielding microscopic cellulose particles. The fine white powder was washed with water, dilute acetic acid, and finally with water until neutral. After washings with alcohol and ether, the material was allowed to air-dry at room temperature. The fluffy white material was then pulverized by light grinding in a mortar.

E. *Preparation of cellodextrin* (*Biosan*). Cellodextrin was prepared according to the procedure of Hess and Friese (11). Analysis of this material confirmed the observation (8) that it has a chain length of from eight to twelve glucose anhydride units.

METHOD EMPLOYED IN THE DISPERSION OF PLANT FIBERS IN CUPRAMMONIUM SOLUTION

The method employed in the dispersion of the various samples listed in Tables I and II, and Figure 1, was essentially the same as that described by Clibbens and Geake (2).

TABLE II

VARIATION OF PARTICLE COUNT WITH CONCENTRATION OF DIFFERENTLY TREATED PLANT FIBERS DISPERSED IN CUPRAMMONIUM SOLUTION

Concentration, per cent	Theoretical number* of particles in volume 2.02×10^{-9} cc.	Number of particles observed in volume of solution 2.02×10^{-9} cc.				
		Cellodextrin (Biosan)	Cottonseed fibers		Wood pulp (Kipawa)	Kiered and bleached cottonseed fiber
			Treated with concentrated HCl (d. 1.18) at 25° C.	Treated with H_2O_2 in presence of 4% NaOH at 100° C.		
0.00	0.0	0.0	0.0	0.0	0.0	0.0
0.25	3.5	0.0	2.8	2.8	3.6	3.4
0.50	7.0	1.8	6.3	5.5	5.7	7.5
1.00	14.0	3.2	13.8	9.4	8.8	13.2
2.00	28.0	3.7	—	—	—	22.2

* Calculation made on basis of uniform cellulose particle, $1.1 \times 1.5\mu$, with density 1.5.

Samples of the various cellulosic materials were taken of such a weight as to yield dispersions of the specified percentage actual cellulose concentrations for each of the calibrated viscometers. The material was finely divided, and, in the case of cottonseed fibers, was cut across with scissors into lengths not greatly exceeding 1/16th of an inch. The samples were conveniently weighed on a small watch glass and quantitatively transferred to the viscometers. A moisture content of 4 per cent was assumed, previous experiments having shown that this figure was a representative average value.

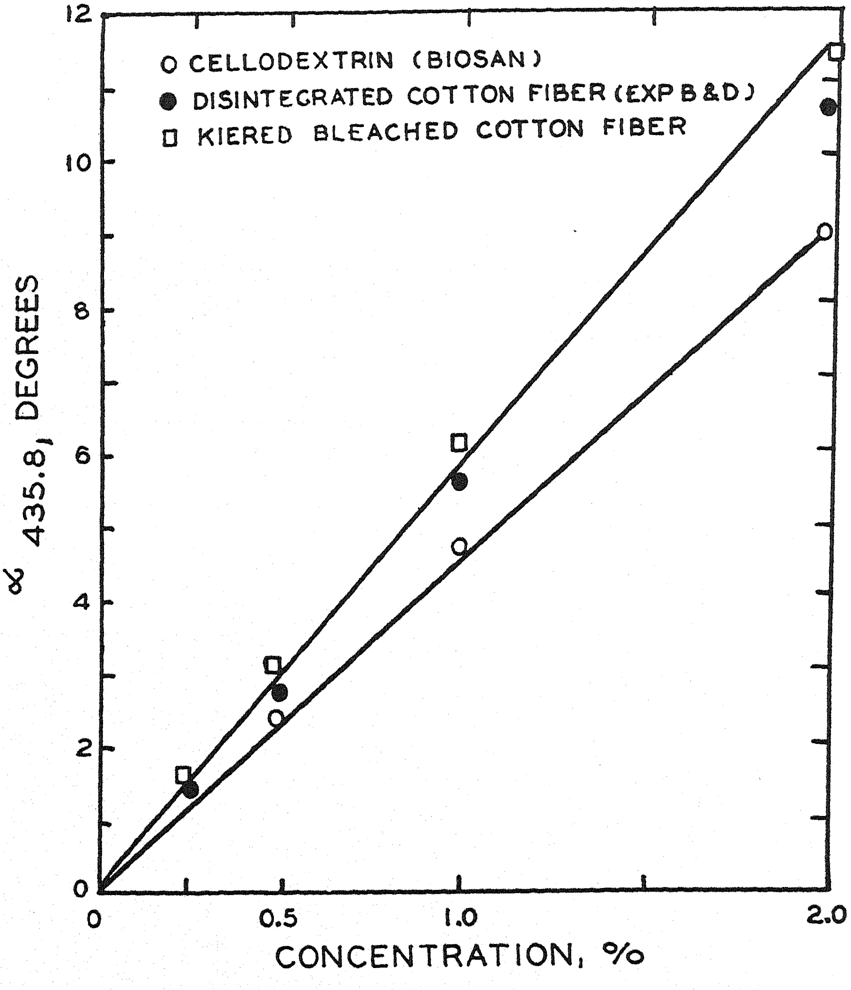

FIGURE 1. Effect of increase in concentration on the optical rotation of variously treated cottonseed fibers dispersed in cuprammonium solution.

A small amount of the cuprammonium solution was drawn up into the capillary end of the viscometer and closed off by folding over the attached rubber tube. The tube was fixed in an upright position and 0.7 cc. of clean mercury run in from a 1 cc. pipette, and the fibrous material transferred to the viscometer by means of small tweezers and tapping the conical-shaped paper which holds it. The tube was then half filled with cuprammonium solution and the sample stirred into the solvent for a few seconds to remove entangled air. The viscometer was then filled with the solvent to within an inch of the top, the rod being washed at the same time. Finally, the viscometer was completely filled with cuprammonium solution and the stopper inserted, so that the excess liquid, displacing all air, overflowed through the top capillary and rubber tube, which was then folded over and made tight with a rubber band; the ground glass stopper was fastened in place by use of steel springs. The viscometer was securely fastened in a box and placed in an apparatus constructed so as to make four revolutions per minute. The end over end motion of the instrument and the resulting motion of the mercury produces an agitation in the liquid which was continued until a homogeneous dispersion was obtained. A uniform period of 18 hours was sufficient for complete dispersion of the variously treated plant fibers. The mixture was maintained at a temperature of 4° C. during dispersion by placing the whole apparatus in a cold room thermostatically controlled.

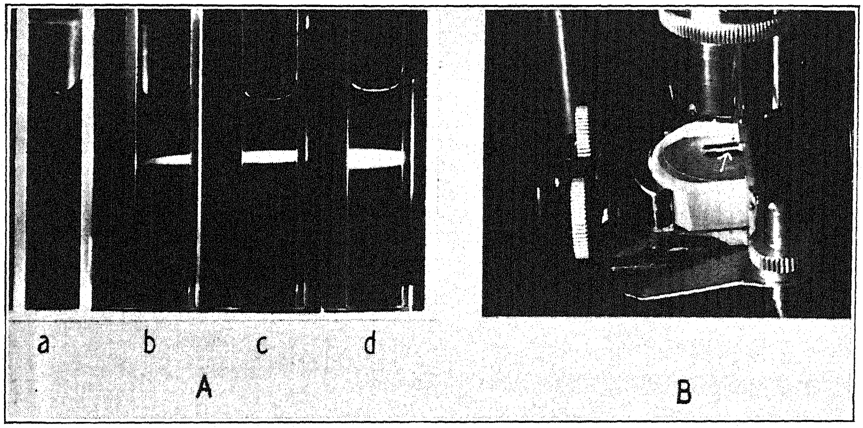

FIGURE 2. (A) Effect of increase in concentration of cellulose dispersed in cuprammonium solution on the Tyndall phenomenon: (a) cuprammonium solution, control; (b) 0.5%, (c) 1.0%, and (d) 2.0% dispersions of disintegrated cottonseed fibers (Exp. B) in cuprammonium solution. (B) Tyndall phenomenon as seen in the micro cell when placed on the stage of the slit ultra microscope.

To measure the viscosities of the resulting dispersions, the viscometers were removed from the box and immersed (2) in a thermostat at 25° C. After standing for 30 minutes, the stopper was removed from the viscometer and the solution allowed to flow freely through the capillary. The time in seconds necessary for the liquid meniscus to fall from the top to the bottom ring was noted with a stop watch.

In determining the optical rotations and particle counts (Tables I, II, and Fig. 1) after viscosity measurements, the dispersions were stoppered and centrifuged (1800 r.p.m.) to remove any entangled mercury droplets. The apparently clear homogeneous dispersions thus obtained were transferred to the polarimeter tube and the micro cell of the ultra microscope and the optical rotations and particle counts determined.

EFFECT OF INCREASING THE CONCENTRATION OF DISINTEGRATED COTTONSEED FIBERS DISPERSED IN CUPRAMMONIUM SOLUTION ON THE TYNDALL PHENOMENON

A typical example of the effect of increasing the concentration of the variously treated plant fibers in cuprammonium on the Tyndall phenomenon is shown in Figure 2 A. Disintegrated cottonseed fibers (Exp. B above) were dispersed in cuprammonium solution in the manner previously described, and a strong beam of light focused into the solutions.

METHOD USED IN MAKING PARTICLE COUNTS ON CUPRAMMONIUM DISPERSIONS OF PLANT FIBERS USING THE SLIT ULTRA MICROSCOPE

Refinement of the method used for observing the Tyndall phenomenon, by use of the slit ultra microscope (Fig. 2 B), enables one to arrive at a quantitative estimate of the number of microscopic cellulose particles present in a definite volume of the dispersions. The cuprammonium dispersions of the various samples were introduced into the micro cell and sealed immediately with a cover glass to prevent evaporation and formation of hydrated copper oxide. The light was then properly focused into the cell and the number of particles in a unit area counted. The average of ten counts at various positions of the field was taken, and this value checked similarly by an independent observer. Controls were run on the cuprammonium solution alone and corrections applied to the average particle counts. Cuprammonium solutions containing more than one microscopic particle per ten unit volumes were rejected. Knowing the focal depth, and the calibration of the eyepiece micrometer for the microscopic lens system used, the unit volume in which the particle counts are made can be calculated. The dimensions of the unit volume were: focal depth $(2.4\mu) \times$ unit area on eyepiece micrometer $(29 \times 29\mu) = 2018.4\mu^3$, or 2.018×10^{-9} cc. The results obtained are recorded in Table II.

To calculate the theoretical number of particles that should be present in the unit volume at a definite concentration, it is necessary to know, first, the average volume of the cellulose particle and second, the total volume of the cellulose dispersed in the solution. The volume of the cellulose particle, V_1, may be calculated from the dimension $1.1 \times 1.5\mu$, by substituting in the equation for the volume of a prolate spheroid, $V_1 = 4/3\pi ab^2$, where a and b are the major and minor semi-axes, respectively. Thus, $V_1 = 4.189 \times 0.75\mu \times (0.55\mu)^2 = 9.95\mu^3$, or 9.5×10^{-13} cc. For a 0.5 per cent (0.5 g. cellulose in 100 cc.) dispersion of purified cottonseed fiber the actual cellulose volume, $V = \frac{0.5 \text{ g.}}{1.5, \text{ density of cellulose } (23)} = 0.33$ cc. In the unit volume, 2.018×10^{-9} cc. there would accordingly be a cellulose volume, V_2, of 6.66×10^{-12} cc. The theoretical number of cellulose particles, N, in the unit volume is obtained from the formula $N = \frac{V_2}{V_1}$. Thus, $N = \frac{6.66 \times 10^{-12} \text{ cc.}}{9.5 \times 10^{-13} \text{ cc.}} = 7.0$, for a 0.5 per cent solution[3]. The corresponding values for other concentrations may be calculated in a similar manner.

EFFECT OF INCREASING THE CONCENTRATION OF VARIOUSLY TREATED COTTONSEED FIBERS IN CUPRAMMONIUM SOLUTION ON THE OPTICAL ROTATION

The experiments were carried out essentially in the manner described by Hess *et al.* (13, 14), with the exception that cuprammonium solution containing 15 g. of copper, 240 g. of ammonia, and 1 g. of sucrose per liter was employed. Dispersion of the variously treated cottonseed fibers was carried out as described above. The results obtained are plotted in Figure 1.

ATTEMPT TO REMOVE THE MICROSCOPIC CELLULOSE PARTICLES OBSERVED IN CUPRAMMONIUM HYDROXIDE DISPERSIONS OF PLANT FIBERS BY FILTRATION

An attempt was made to remove the cellulose particles from a 0.5 per cent cuprammonium dispersion of disintegrated cottonseed fibers (Exp. B, above) by filtering through a Jena sintered glass pressure filter, porosity 5/3. Although the filtration time of the dispersion was much greater than that of the cuprammonium solution alone, no residue was obtained on the filter disc. Slit ultra microscopic observation of the filtered dispersion, in the manner described above, showed no diminution of the number of particles present. Since the largest pore diameter of the filter disc was 1.82μ, free cellulose particles ($1.1 \times 1.5\mu$) would be expected to pass through. Efforts to obtain a sintered glass filter of finer pore dimensions are being made.

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[3] Due to the variable values reported for the true density of cellulose (23) and allowing for possible slight error in the dimensions of the cellulose particle, the values of N cannot be considered absolute, but do not exceed a possible correction of more than ±20 per cent.

DETERMINATION OF THE "SATURATION POINT" OF DISINTEGRATED COTTONSEED FIBERS IN CUPRAMMONIUM SOLUTION

The possibility of the existence of a mass action equilibrium between the cellulose-copper complex and cellulose in cuprammonium dispersions has been previously considered (12, 13, 19, 26). In the present experiment disintegrated cottonseed fibers (Exp. B and D above) have been used to determine the point at which the cuprammonium solution could no longer cause permanent dispersion. When the concentration was increased beyond 4.5 per cent, the excess undispersed fiber fragments could be centrifuged unchanged (1800 r.p.m.) from the permanently dispersed phase. The clear supernatant dispersion was carefully removed by decantation and poured into a large excess of cold dilute hydrochloric acid solution. After standing a few minutes the coagulated product was removed by centrifuging, and washed with distilled water until neutral, and air-dried at room temperature. The solid phase remaining in the original centrifuge cup was washed with concentrated ammonium hydroxide solution until the blue copper ions had been completely removed and then with water until neutral. X-ray diffraction patterns of the latter and former phases were then obtained (Fig. 3 A and B).

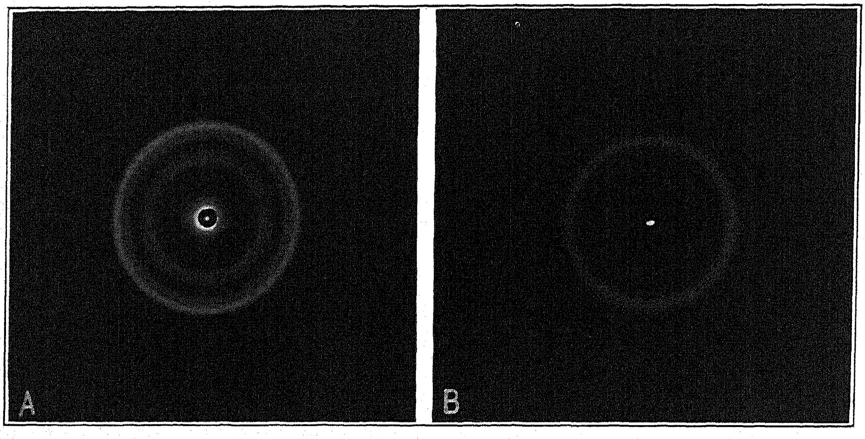

FIGURE 3. (A) X-ray diffraction pattern of the excess undispersed phase recovered from a 5.0% dispersion of disintegrated cottonseed fibers (Exp. D) in cuprammonium solution, showing the characteristic native pattern. (B) X-ray diffraction pattern of the permanently dispersed phase, after coagulation with dilute acid, of a 5% dispersion of disintegrated cottonseed fibers (Exp. D) in cuprammonium solution, showing a typical mercerized cellulose pattern.

DISCUSSION

The two general methods employed for the gradual chemical disintegration of plant fibers are: first, the action of dilute acids, and second, the action of mild oxidizing agents. The disintegration of plant fibers in this manner is accompanied by a lowering of the cuprammonium viscosity (Table I) which has been previously interpreted as being due to a decrease in the chain length of the cellulose molecules. Based on the dual structure of plant fibers, however, a new interpretation is now placed on this behavior. Since the crystalline phase, or cellulose particles, remains largely intact by these treatments, it follows that the intercrystalline "cementing" material is the fiber phase most easily extracted or modified. The conclusion may also be drawn that this unstable, reactive phase of native plant fibers is largely responsible for the high viscosity when dispersed in cuprammonium solution. This interpretation is somewhat similar to that of Joyner (16) who postulated the existence of a cellulose A with high and a cellulose B with low viscosity in native plant fibers.

The high optical activity of purified plant fibers colloidally dispersed in cuprammonium solution was considered by Hess *et al.* (12, 13, 14) to be due to the uniform formation of an asymmetric complex cellulose-copper compound $[(C_6H_7O_5)Cu]_2[Cu(NH_3)_4]$. It was further shown that with plant fibers of low viscosity there was no relation between optical activity and change in cuprammonium viscosity. The present work extends this conclusion to the entire viscosity range possible with cottonseed fibers (Table I). Thus, the cellulose particle, or crystallite, seems to be responsible for the high optical activity, for as the viscosities of the cuprammonium dispersions are lowered, indicating removal of the intercrystalline "cementing" material, the rotations remain constant. The high optical activity of cottonseed fibers of high viscosity shows that either the rotation of the intercrystalline-copper complex is very similar to that of the cellulose particle-copper complex, or that the intercrystalline phase is present in quantities so minute as to escape detection.

Although many workers have recognized the fact that purified plant fibers disperse in cuprammonium to give colloidal solutions (15, p. 138), this property has usually been attributed to the supposed great length of the cellulose molecules. Staudinger (24) refers to molecules whose size exceeds the typical molecular dimensions (molecular weight above 10,000) as being eucolloids or molecular colloids. Accordingly, cellulose, meaning a purified plant fiber, is often considered a one-dimensional eucolloid (1, p. 105). As one would thus expect on the basis of particles of microscopic dimensions, or of molecular colloids, the Tyndall phenomenon is definitely exhibited by purified plant fibers dispersed in cuprammonium solution (Fig. 2 A). When such dispersions are carefully examined in strong light at high magnification with the slit ultra microscope (Fig. 2 B), however, discrete microscopic particles may be seen. Further, examination of cuprammonium dispersions of disintegrated cottonseed fibers, the viscosity of which had reached a constant low value (Table I), showed practically the theoretical possible number of cellulose particles ($1.1 \times 1.5\mu$) to be present (Table II). The variable deviations of the observed number of particles from the theoretical with increasing concentration is due to the difficulty of obtaining an absolute estimate from relatively few counts. When the number of counts on the samples was increased, the average value more closely approached the theoretical. The significant fact, however, is the trend toward an increase in particle count with increase in dispersed fiber concentration. Due to the Brownian movement of the dispersed particles and the convection currents set up in the micro cell by heat from the light beam, an accurate count is difficult to obtain on more than one per cent concentrations of plant fibers of low viscosity. With high viscosity plant fibers, however, such as kiered and bleached cottonseed fibers, motion in the dispersion is greatly reduced. Wood pulp fibers dispersed in cuprammonium solution showed the same particle count tendency as cottonseed fibers, indicating that both are composed of the same elementary cellulose units.

Inasmuch as cuprammonium dispersions of plant fibers contain microscopically visible particles and possess high optical activity, it would appear that the organized cellulose units must be able to both transmit and reflect light. In ordinary light without high magnification, these dispersions appear as true solutions due to even lighting from all directions of the transparent cellulose particle-copper complex.

Cuprammonium dispersions of a cellodextrin such as "Biosan" are slightly less optically active than treated plant fibers (Fig. 1). Dilute dispersions are optically void when examined in the slit ultra microscope, but upon increasing the concentration a few particles are observed (Table II). It appears then that this cellulose-like substance is dispersed beyond the limits of microscopic visibility. The conclusion may thus be drawn that regardless of the state of aggregation of the cellulose-copper complex, the optical rotation is of the same order of magnitude.

The existence of a definite equilibrium, between the permanently dispersed cellulose particle-copper complex phase and the excess undispersed cottonseed fiber phase at concentrations exceeding four and one-half per cent in cuprammonium solution containing 15 g. of copper and 240 g. of ammonia per liter, is shown by the fact that saturation is reached at this point. The saturation point has only been observed with dispersions of disintegrated cottonseed fibers (Exp. B and D), inasmuch as the viscosity of native cottonseed fibers is much too high to make the point distinguishable. The excess undispersed phase may be almost entirely recovered unchanged upon centrifuging cuprammonium dispersions containing more than four and one-half per cent of disintegrated cottonseed fibers. X-ray diffraction analysis[4] shows the excess undispersed phase, thus recovered

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[4] Prior to the author's independent observation, W. A. Sisson of this laboratory noted this apparent equilibrium from a qualitative examination of cuprammonium dispersions of cellulose particles.

from a more than saturated cuprammonium dispersion, to be native cellulose, whereas the permanently dispersed phase upon coagulation gives a mercerized or hydrated cellulose pattern (Fig. 3 A and B). Since the undispersed phase is not combined with the copper or ammonia in any way, it appears that the intermediary formation of the cellulose-copper complex is necessary for the transformation of native cellulose into the mercerized form, or that copper complex formation and dispersion of cellulose occur simultaneously.

SUMMARY

1. The successive lowering of the cuprammonium viscosity of native cottonseed fibers by the action of dilute acids or mild oxidizing agents is not accompanied by a change in the optical activity of the resulting dispersions.

2. It then follows that the optical activity of plant fiber dispersions in cuprammonium solutions is dependent upon the formation of a cellulose particle-copper complex.

3. Quantitative examination of variously treated plant fibers dispersed in cuprammonium solutions, using the slit ultra microscope, reveals the presence of approximately the theoretical number of cellulose particles ($1.1 \times 1.5\mu$).

4. Further evidence that cellulose-copper compound formation precedes dispersion of cellulose in cuprammonium solution is presented.

5. Visible cellulose particles ($1.1 \times 1.5\mu$), observed by Farr and Eckerson in young cottonseed fiber cytoplasm and disintegrated mature cottonseed fibers, have now been observed in dispersions of plant fibers in cuprammonium solution. It is proposed that the behavior of plant fibers when dispersed in cuprammonium solution is attributable to properties of the crystalline microscopic cellulose particle in conjunction with the intercrystalline fiber phase.

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BEHAVIOR OF THE CELL MEMBRANE OF THE COTTON FIBER IN CUPRAMMONIUM HYDROXIDE SOLUTION

WANDA K. FARR[1]

The first *recorded* observations of the remarkable action which ammoniacal solutions of cupric oxide exert upon cotton fibers are in the unpublished laboratory notes of John Mercer. He found that the fibers were soluble in a liquid obtained by dissolving hydrated cupric oxide to saturation in ammonia of sp. gr. 0.920 and diluting with three measures of water. On neutralizing with sulphuric acid, the organic matter was precipitated and later freed from copper by an excess of the acid. When fibers were similarly treated for one minute, their surfaces were changed to a gummy or mucous consistency. The effect of temperature was determined by placing a portion of the cuprammonium solution outdoors on a frosty night; another portion was warmed to 100° F. When cotton cloth was dipped into each of these for ten minutes, the cloth in the cold solution was acted upon very powerfully and that in the warm solution was acted upon scarcely at all.

Mercer's experimental data were made available by his family to Edward H. Parnell (26) and were used in the preparation of his book, "The Life and Labours of John Mercer," published in 1866. The exact year in which Mercer's observations of the action of cuprammonium hydroxide upon cotton fibers were made is not given by Parnell. The relation of this work to other dated experiments suggests that it was about 1851.

The first *published* record of the use of cuprammonium hydroxide as a solution-medium for plant fibers came from the laboratory of Edward Schweizer (33) in 1857. The observation that this solution dissolves plant fibers at ordinary temperatures was apparently original with him. He described the process in which the fiber first gelatinized and then disappeared into a viscous fluid which could be stirred with a glass rod. If a small amount of fluid were used, some of the fiber mass remained; if an excess of the solvent, a clear blue liquid resulted. This mixture, thinned with water, filtered, and acidified, produced a voluminous precipitate. Schweizer pointed out that this precipitate, although completely disorganized, was not different in its chemical properties from cellulose. He also found that flax fibers and wood fibers behave in a similar manner and he expressed surprise that starch, so closely related in chemical composition, did not dissolve in cuprammonium hydroxide.

The observations of Cramer (9) upon the effect of cuprammonium

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[1] Cellulose Department, Chemical Foundation, Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York.

hydroxide upon plant cell membranes, starch, inulin, the cell nucleus and the primordial utricle appeared in 1858. Both Schweizer and Cramer observed the now familiar globe-like swelling behavior of single fibers. Both reported that the fibers in the region of the constricting bands are strongly doubly refractive and that the globes are non-doubly refractive. They found also that after removal of the cuticle there are no globe-like formations during the swelling of the fiber and that the reaction itself is much more rapid. Cramer reported the same type of globe-like swelling in cotton fibers after treatment with H_2SO_4. He also found that starch could be enlarged 2-1/2 to 3 diameters if treated with chilled cuprammonium hydroxide.

During the same year Peligot (27, 28), having heard of the work of Schweizer, attempted to repeat the experiments in France. He had no details of Schweizer's method of preparation of the cuprammonium hydroxide solution but, by treating copper with ammonia, finally obtained a liquid which gelatinized cotton fibers when the mass was agitated. He also observed that when the fibers had completely disappeared, the mixture was not perfectly limpid, although this was difficult to appreciate because of the blue coloration. Addition of acid brought about immediate formation of a white precipitate which he described as unaltered cellulose.

Charles O'Neill (25), in 1864, followed the stages of swelling of the cotton fiber in both weak and strong solutions of cuprammonium hydroxide, confirmed the earlier reports of the insolubility of the external membrane, and described the final stages of swelling in which the cellulose appears as a homogeneous mass while the empty cuticular membrane remains nearly unacted upon.

Eugène Gilson (19) published a more comprehensive chemical and microscopic analysis of the behavior of plant cell membranes in cuprammonium hydroxide in 1893. His interest in the problem was stimulated by the observations and interpretations of Behrens (6), Tschirch (35), Reiss (30), Schulze, Steiger, and Maxwell (32), and Schulze (31) which had been published during the preceding decade. A study of the first three papers of this group serves to make clear the confusion which existed at that time concerning the nature of the plant cell membrane. As Gilson explained (19, p. 397), certain workers believed that the membrane consisted of a single substance which was itself a chemical individual; others believed that the membrane consisted of many physical modifications of cellulose; still others designated under the name cellulose substances which were very different chemically. Schulze and his collaborators reported the existence, in the plant cell membrane, of hydrates of carbon of many different types. They reserved the name cellulose for the particular membrane components which dissolved in cuprammonium hydroxide, were not attacked by weak acids, and which were colored blue with sulphuric acid and iodine and with chlor-zinc-iodide. According to them there were several of these true celluloses which were the anhydrides of dextrose, galactose, arabinose, xylose, and mannose. They classified as hemicelluloses the carbohydrates of the membrane which were soluble in weak acids. They repeatedly referred to the amorphous nature of cellulose and sometimes designated it as an example of an uncrystallizable body.

Gilson was not convinced that this belief was correct and attempted to obtain crystalline cellulose from a solution of cellulose in cuprammonium hydroxide. He studied the reactions both of tissue sections in which the entire cells could be observed, and also of fragments of cell membranes. In the interior of the cells which he had treated with cuprammonium hydroxide and subsequently washed with water, he reported the presence of an amorphous mass which gave the reaction of cellulose. By treating larger quantities of plant tissues with weak alkali and then with Schweizer's reagent, a viscous mixture was obtained which he allowed to remain until the ammonia had evaporated. The residue was washed with dilute hydrochloric acid to remove the copper, then with distilled water, and finally dried. Both the intracellular formations and the powder residues were found, by means of the microscope, to consist of what he termed "sphaerocrystals." His illustrations show structures which subsequent workers have not been able to produce in these same materials. Johnson (20) repeated Gilson's work in 1895. After treatment with Schweizer's reagent, material which he identified as cellulose was readily obtained in the cells of *Beta*, *Dahlia*, *Lactuca*, *Typha*, *Ceratozamia*, *Equisetum*, and *Chara*. Although Gilson had worked most intensively upon the cells of the beet root, he had used about fifty other plants. These were mostly angiosperms but included also *Chara*, *Spirogyra*, *Mucor*, *Agaricus*, six mosses, and two gymnosperms. In all of the cells, except those of the fungi, satisfactory "crystals" of cellulose were obtained by the treatment with cuprammonium hydroxide. Johnson obtained a blue color with sulphuric acid and iodine and chlor-zinc-iodide in cell membranes of *Mucor*, *Agaricus*, and *Saprolegnia*, as well as the fungal portion of several lichens, but was not able to produce the characteristic reaction with Schweizer's reagent in the cells of these plants. In all, these workers had studied a sufficiently representative number of plants to indicate that the cellulose behavior which they described was fairly uniform throughout the plant kingdom. Johnson remarked that, after keeping the sections of the beet root in Schweizer's fluid for 20 days, the crystallized cellulose was as plentiful in the cells as at first. There was no evidence of diffusion in the dissolved state. Examination of these treated tissues in polarized light led to the statement that the crystalline material was non-doubly refractive. Johnson expressed doubt, therefore, that the structures which they observed were really true crystals. These observations are of special interest in relation to the data to be presented in this paper. Both Gilson and Johnson believed that the cellulose in the membrane had dissolved in Schweizer's reagent and recrystallized from this solution. Bütschli (7) contemporaneously published photomicrographs of the cotton fiber in cuprammonium hydroxide. He repeated Gilson's technique and, in the swollen membranes, found evidence of the "honeycomb" structure which he believed to be the fundamental pattern of many organic and inorganic materials. The data and interpretations of these three workers have been somewhat confusing and have contributed little conclusive evidence as to the physical nature of the substances with which they were dealing.

The important contributions concerning the crystalline nature of cellulose through the medium of X-ray diffraction made their first appearance in the paper of Nishikawa and Ono (24) in 1913. This, and the large number of subsequent papers, has established the fundamental crystalline nature of cellulose in the sense of orderly molecular arrangement. These X-ray diffraction data have been interpreted by other workers (14) in terms of the submicroscopic cellulose "micellae" of Nägeli (23) and their orientation as described by Ambronn (2). Extensive correlations have been made also between the hypothetical cellulose micellae and the viscosities of cell membranes of various types in cuprammonium hydroxide. The concern of the late nineteenth century workers over the complexity of these cell membranes as related to their chemical and physical properties has not entered into the interpretations of these more recent investigations. "Cellulose," "Purified Cotton," "Purified Wood Pulp," etc., are used synonymously. Reactions of the fibrous masses are interpreted as reactions of a single chemical compound—cellulose.

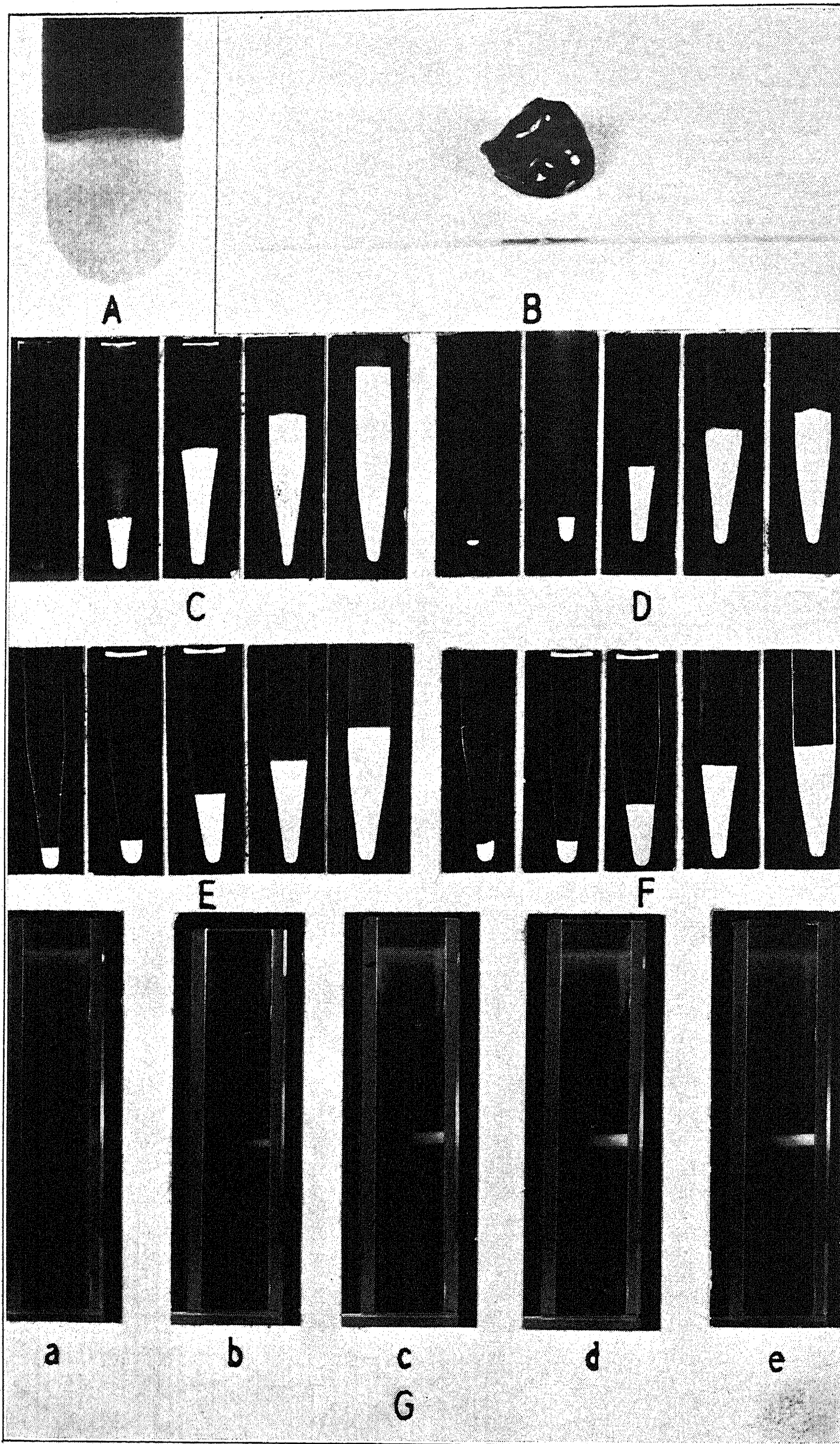
The studies of orientation of the cellulose in the membrane by means of polarized light and X-ray diffraction have been supplemented more recently by direct observations of fibril orientation in ordinary light (29). The degree of correlation in the results obtained with these three methods has established Ambronn's original conclusions concerning orientation beyond any reasonable doubt. It must be kept clearly in mind, however, that in these studies of orientation, the X-ray diffraction and polarized light analyses have furnished no direct evidence as to the size of the crystallite itself. Certain diffraction phenomena have been correlated with variations in viscosity of mixtures of cell membranes and cuprammonium hydroxide and other reagents, however, in the postulation of a wide range of micellar sizes.

The exact form and size of the crystallite, as well as its physical and chemical reactions, have been the source of controversy in many allied fields of research. Physiologists have been constantly reminded of the fact that a plant cell membrane made up entirely of submicroscopic crystalline micellae could not exhibit the observed behavior during swelling. These doubts have been augmented by the observance of profound changes in cell membranes after treatment with reagents which could not be shown to have affected the cellulose itself. A cell membrane may thus lose all of its natural physical characteristics, be resolved into a fine white powder, and during the process the cellulose X-ray pattern shows no evidence of change except the transition from regular to random orientation (17). The microchemical reactions of the cellulose are also unaltered (16).

A new approach to the problem of the nature of the cellulose crystallite was made in our laboratory, in 1930, through dissections of fresh membranes and mild chemical treatments of dried membranes. The process of membrane formation was studied in many types of living plant cells from various parts of the plant kingdom. In 1932 we observed large numbers of uniform-sized ellipsoid particles in the cytoplasm of these living cells (15). They are remarkably stable physically and, although diminutive in size ($1.5 \times 1.1\mu$), lend themselves readily to both optical and microchemical analyses. They are definitely doubly refractive in polarized light; the refractive indices of the moist particles from the living cytoplasm of the cotton fiber are 1.565 lengthwise and 1.530 crosswise; they swell and turn blue in the presence of sulphuric acid and iodine; their combustion analysis yields: $C = 44.32$, $H = 6.30$; their X-ray diffraction pattern is that of native cellulose (34); and when treated with 18 per cent NaOH they give the pattern of mercerized cellulose. In brief, these ellipsoid particles show the properties characteristic of cellulose and were designated by us "cellulose particles."

Although separate and in short chains in the interior of the living cell, the cellulose particles, during the period of wall formation, appear in the outer regions of the cytoplasm arranged end to end in orderly, single rows and finally become so closely appressed that they lose their optical identity in the formation of the well known cellulose *fibril*. The fibrils were removed from the living cells for more careful examination. It then became clear that each particle in the fibril is coated with a viscous material which functions as a cementing material, holding the particles together to form the fibril and the fibrils, layer upon layer, to form the membrane lamellae (12).

Membranes of moist cotton fibers from the unopened boll can be disintegrated into fibrils and particles by mechanical treatment alone. The membranes of mature, dried fibers require treatment with weak chemical reagents for a long period of time or strong reagents for a shorter period of time to bring about a similar degree of membrane disintegration. As the treatment proceeds the cementing material surrounding the particles is observed microscopically to swell and in so doing to push the particles farther apart. Continued treatment brings about the removal of the cementing material by the solvent and the cellulose particles remain in the form of a fine white powder. If a weak reagent such as 0.5 to 2.0 per cent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$(NH_4)_2C_2O_4.H_2O$ is used, the fraction of the cementing material soluble in this liquid may be recovered by the addition of approximately twice the volume of acidified alcohol to the filtrate. If the procedure is carried out under the proper conditions of pH, temperature, and concentration of the filtrate, the precipitate is in the form of a dense, easily recoverable, colorless gel. Its analysis, according to the current technique for uronic acid determination (10), places it in the category of pectic substances. The most significant fact is that *membrane disintegration* is brought about without any measurable indication of *cellulose degradation*. Physically and chemically the cellulose particles so obtained are no different from those which we observe in the cytoplasm of the living cells.

Through the application of these techniques, cellulose particles, as well as the pectic fraction of the cementing material extracted by ammonium oxalate, were soon available in considerable quantities for further experimentation. The study of the behavior of separated cellulose particles in solutions of cuprammonium hydroxide was, obviously, one of the most interesting and promising fields. With this degree of purification, the highest viscosities per unit weight of cellulose in cuprammonium yet obtained were to be expected. It was of even greater importance that we should learn whether or not the separated cellulose particles could be dissolved in cuprammonium hydroxide and reprecipitated in their original state from the solution. When separated cellulose particles were added to a solution of cuprammonium hydroxide prepared according to the recommendation of the Committee of the American Chemical Society on the viscosity of cellulose, the viscosity of the mixture was only slightly higher than that of the cuprammonium solution itself. Although the preparations appeared to be free of undissolved material, microscopic examination revealed that the particles were in a state of dispersion unchanged in shape and size. The following report covers a study of the behavior of the cellulose particles in this standardized solution of cuprammonium hydroxide and contributes the additional information that the viscosity-producing

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FIGURE 1. (A) Cellulose particles separated by treatment with HCl (sp. gr. 1.19), agitated for 10 minutes with an electric stirrer in the standard solution of cuprammonium hydroxide, settle to the bottom of the tube during a 48-hour period. Temperature of cuprammonium treatment—4° C. (B) Pectic material extracted from cotton fibers with 0.5 per cent ammonium oxalate produces a stiff gel in cuprammonium hydroxide at any concentration over 5 g. per 100 cc. of solution. (C) Cellulose particles similar to those used in (A) added to cuprammonium hydroxide in quantities of 0.05, 0.1, 0.5, 1.0, 2.0 g. per 100 cc. photographed after 48 hours. Temperature 4° C. (D) The series of tubes shown in (C) after centrifuging. (E) Cellulose particles in similar quantities in water photographed against a black background after 48 hours. (F) The series of tubes shown in (E) after centrifuging. (G) a, Absence of the Tyndall beam in the cuprammonium solution alone; b, c, d, e, Tyndall beams of increasing brightness with 0.25, 0.5, 1.0, and 1.5 g. of cellulose particles per 100 cc. of cuprammonium solution.

power of the cotton fiber in cuprammonium hydroxide is located in the cementing material which is intimately associated with the cellulose particles in the formation of the fiber membrane.

MATERIAL AND METHODS

The cotton fibers used were grown for us in the experimental plots of the South Carolina Experiment Station, Clemson College, South Carolina, through the courtesy of Dr. George M. Armstrong. The same variety, *Gossypium hirsutum* L. Strain 4, had been used in the earlier studies of the formation and structure of the plant cell membrane (15).

Cellulose particles were separated from these membranes by two different procedures: (a) treatment with strong HCl (sp. gr. 1.19) at room temperature for a comparatively short period of time; (b) repeated extraction with weak acids and alkalies (2 per cent HCl, 1 to 2 per cent KOH) and with distilled water for a long period of time (about 3000 hours) at 75° C.

The pectic fraction of the cementing material was obtained by precipitation from the filtrate of the ammonium oxalate extraction. Through adjusting the pH of the concentrated filtrate to 2.63 and by allowing it to drip slowly from a separatory funnel into twice its volume of alcohol chilled to a temperature of 4° C., the precipitate was obtained in the form of a stiff gel. It was then easily recovered upon a silk filter, dried, and ground. The samples were purified by repeated dispersions in water and reprecipitated according to the described procedure.

The solutions of cupric oxide in ammonia were prepared according to the recommendation of the Committee of the American Chemical Society on the viscosity of cellulose (3). The copper content was 30 ± 2 g.; the ammonia 165 ± 2 g.; and sucrose in the proportion of 10 g. per liter.

Carefully weighed samples of fibers were added to the cuprammonium solution and agitated for 10 minutes with an electric stirrer. Temperatures were maintained at 4° C. In this length of time at this temperature, the blending of the mixture was sufficiently uniform to flow at a constant rate through the small bore of the viscosity tube. There were no visible fiber fragments in the tube, but in a strong light it was evident to the unaided eye that the solutions were not optically clear. Viscosities were determined at 4° C. in an Ostwald modification of the Poiseuille viscosity apparatus.

RESULTS

Cellulose particles from which the cementing material had been removed by treatment with HCl (sp. gr. 1.19), when added to the A.C.S. standard solution of cuprammonium hydroxide and stirred vigorously at a temperature of 4° C., settled slowly to the bottom of the tube during a period of 12 to 24 hours (Fig. 1 A). When particles were added in increasing

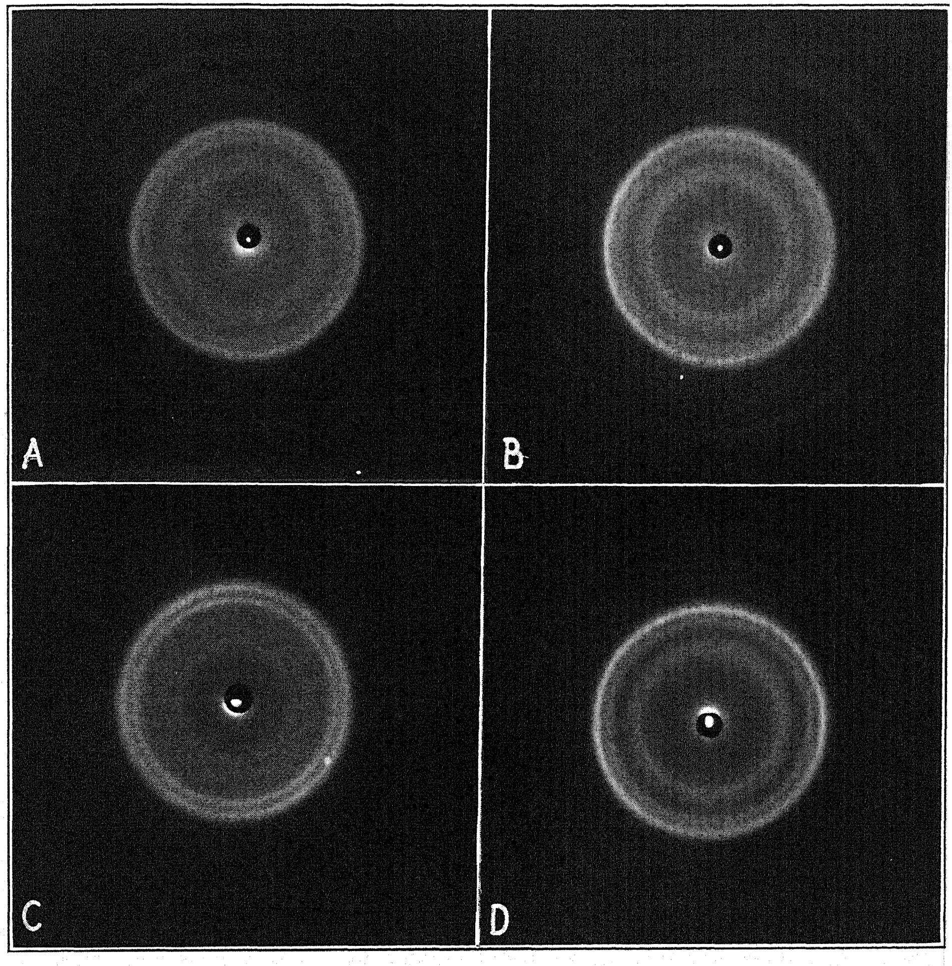

FIGURE 2. (A) X-ray diffraction pattern of cellulose particles from cotton fibers after treatment for 48 hours with the standard solution (American Chemical Society) of cuprammonium hydroxide. (B) A similar sample after three months' treatment with the same reagent. (C) 3 g. of cellulose particles separated by treatment with HCl (sp. gr. 1.19) produce a mixed mercerized and native cellulose X-ray diffraction pattern after treatment for 18 hours in cuprammonium hydroxide. (D) 7 g. of cellulose particles similar to those used in (C) produce a native cellulose X-ray diffraction pattern after treatment for 18 hours in cuprammonium hydroxide.

amounts (0.05, 0.5, 2 g. per 100 cc.), they accumulated in the bottoms of the tubes in proportion to the amount that had been added (Fig. 1 C). These same tubes, centrifuged, are shown in Figure 1 D. Tubes containing equal quantities of cellulose particles in water, photographed against a black background, both before and after centrifuging, are shown in Figure 1 E and F.

The cuprammonium solution was not made viscous by the addition of any quantity of these particles. In Table I the measured viscosity of a solution of 0.50 g. of particles, prepared by separation with strong HCl, was 4 min., 24 sec., as compared with 3 min., 34 sec. for the cuprammonium solution alone. Half of this quantity of untreated cotton fibers under the same conditions had a measured viscosity of 102 min., 50 sec.

TABLE I

VISCOSITIES OF CELLULOSE PARTICLES IN CUPRAMMONIUM HYDROXIDE; TEMPERATURE 4° C.

Sample	Extraction medium	Length of extraction	Extraction temperture	G. per 100 cc. of cuprammonium hydroxide	Viscosity (time)
Cuprammonium hydroxide solution (control)	—	—	—	—	3 min. 34 sec.
Cellulose particles from cotton fibers	HCl (sp. gr. 1.19)	18 hrs.	Room temp.	0.50	4 " 24 "
Cellulose particles from cotton fibers	2% KOH	3661 "	75° C.	0.25	4 " 1 "
Cellulose particles from cotton fibers	H_2O distilled	6081 "	75° C.	0.25	6 " 45 "
Cellulose particles from cotton fibers	2% $(NH_4)_2C_2O_4 \cdot H_2O$	3126 "	75° C.	0.25	14 " 50 "
Cotton fibers (for comparison)	Untreated	—	—	0.25	102 " 50 "

The X-ray diffraction pattern of the cellulose particles from cotton fibers which had settled to the bottom of the tube during a 48-hour period, was that of native cellulose (Fig. 2 A). A similar sample which stood in a sealed tube at 4° C. for 3 months gave, likewise, a native cellulose pattern (Fig. 2 B). Figure 2, C and D, shows the diffraction patterns of two samples in a later series which were prepared from dispersions of 3 g. per 100 cc. and 7 g. per 100 cc. respectively. The lower concentration produced a diffraction pattern of mixed mercerized and native cellulose and the higher concentration a native cellulose pattern. These later samples were prepared for diffraction analysis by evaporation of the ammonia from the dispersion and removal of the copper from the residues with 1 per cent HCl. The first two samples (Fig. 2 A and B) were prepared for diffraction analysis by siphoning off the supernatant liquid before drying and washing the residue. More recent studies of the behavior of cellulose particles in

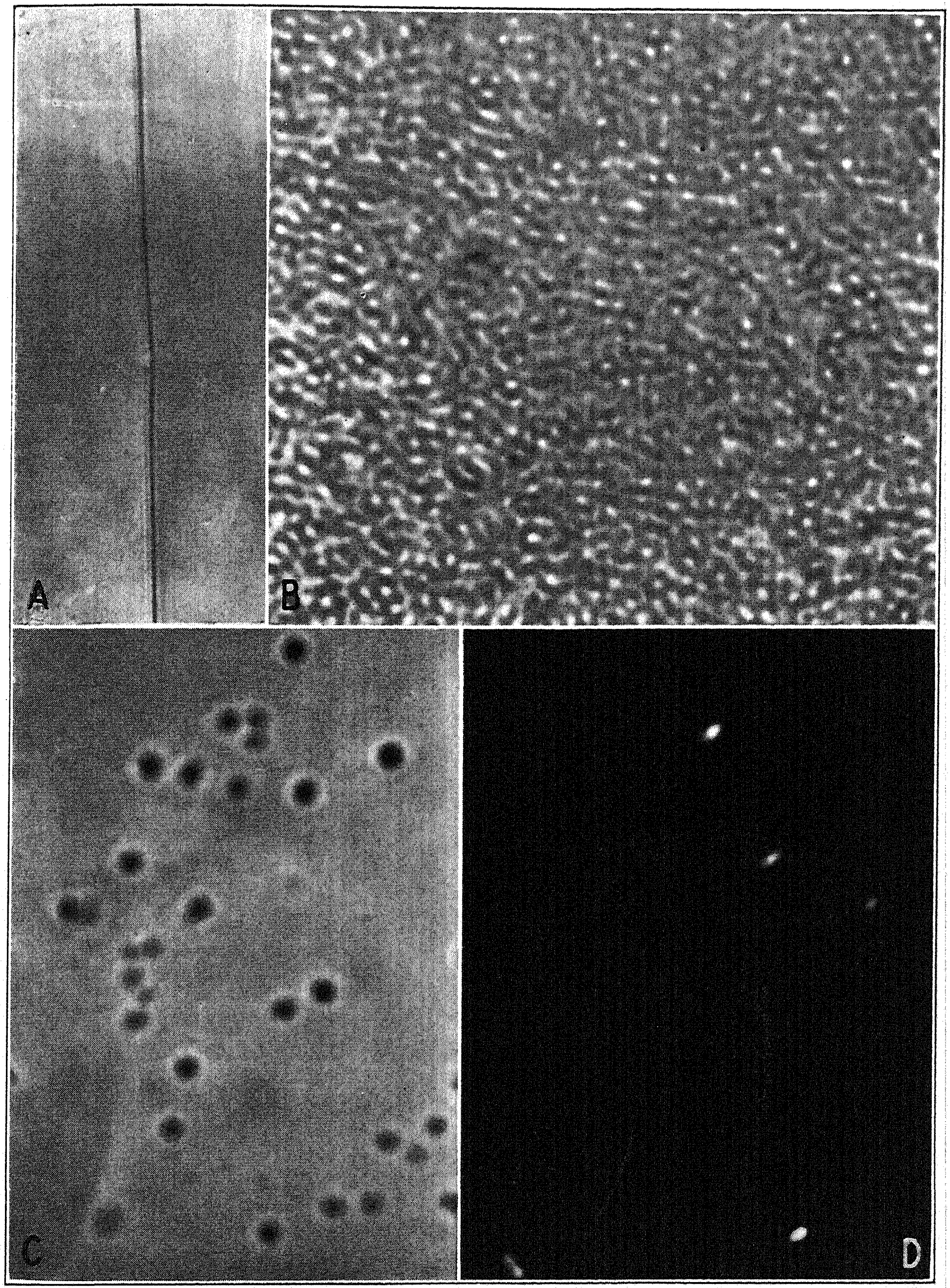

FIGURE 3. (A) The cuprammonium hydroxide solution used in these microscopic studies was optically clear. A strand of glass wool is in focus (×630) (B) Cellulose particles from untreated cotton fibers retain their original shape and size when treated with this reagent (×1640). (C) After treatment with cuprammonium hydroxide, they give the characteristic cellulose reaction to sulphuric acid and iodine (×1640). (D) After treatment with cuprammonium hydroxide, they are still doubly refractive in polarized light (×1500).

the British Standard Solution of cuprammonium hydroxide have thrown some light upon the native and mercerized states of these samples (8). Particles which are truly dispersed have been found by Sisson and Compton to produce a *mercerized* X-ray diffraction pattern; those which gradually settle to the bottom of the tube produce a *native* diffraction pattern. In the presence of a large excess of "native" particles, the "mercerized" particles are not in evidence in the diffraction pattern.

A microscopic examination of the cuprammonium solution itself showed the presence of no undissolved material in the dark blue mixture (Fig. 3 A). A microscopic mount from a well-agitated mixture of cellulose particles and cuprammonium showed the presence of large numbers of cellulose particles apparently unaltered in shape and size (Fig. 3 B). These particles, removed from the cuprammonium solution and treated with strong sulphuric acid and iodine, swelled and were colored blue in the manner characteristic of cellulose particles from the living cell (Fig. 3 C). When properly oriented in polarized light, they were seen to be definitely doubly refractive (Fig. 3 D).

To another series of tubes, the first of which contained cuprammonium hydroxide alone, cellulose particles were added in increasing quantities. They were, in turn, placed in the path of a concentrated beam of light from an arc lamp. No effect of the beam was observable in the tube which contained only cuprammonium hydroxide. The Tyndall phenomenon was definitely present, however, in the tubes to which the cellulose particles had been added, increasing in intensity as the concentration of the particles increased (Fig. 1 G). This observation was carried a step further through examination of the Tyndall beam produced by the cellulose particles in the cuprammonium hydroxide solution by means of the slit ultra microscope[2]. Large numbers of uniform-sized particles were seen in active Brownian movement in the path of the light. Their ellipsoid shape and size identified them definitely with the cellulose particles previously described and found to be visible microscopically by means of ordinary transmitted light in the same cuprammonium hydroxide solution.

The results so far showed that the cellulose particles, freed from cementing material by treatment with strong HCl, do not dissolve to produce the viscosities commonly attributed to cellulose in cuprammonium hydroxide and that large numbers, if not all, of the cellulose particles added could be observed in this solution in a state of dispersion.

Cellulose particles which had been obtained from cotton fibers by long continued and repeated extraction with 2 per cent KOH, 2 per cent $(NH_4)_2C_2O_4 . H_2O$, and even distilled water, behaved similarly in the A.C.S. standard solution of cuprammonium hydroxide. Microscopic examination

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[2] This instrument was made available in 1935 through the courtesy of E. Leitz, Inc., New York.

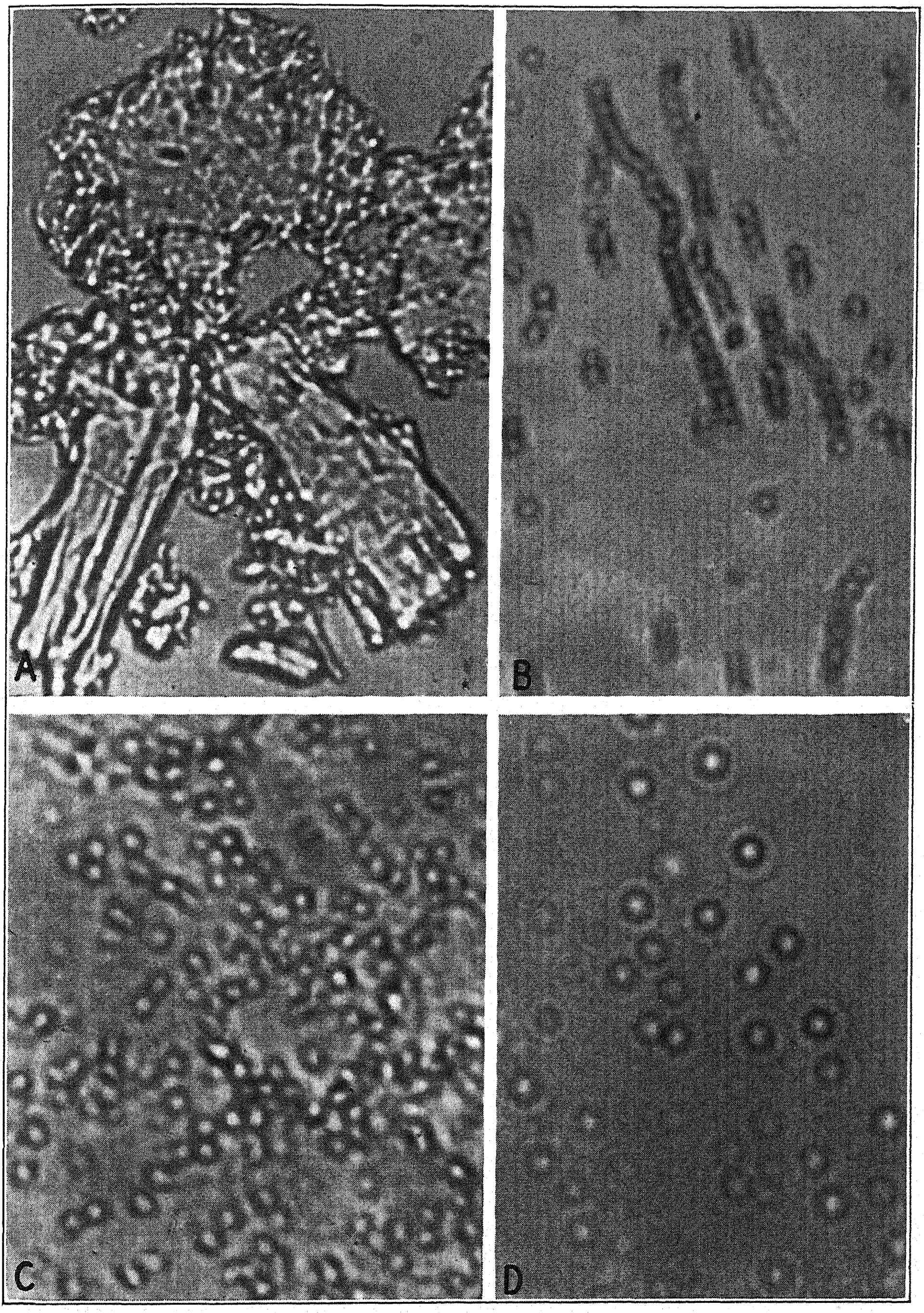

FIGURE 4. (A) Purified cotton linters disintegrate rapidly into fibrils and particles after treatment with HCl (sp. gr. 1.19) (×1100). (B) Fibrils dissected away from the fiber fragment in (A) give the characteristic cellulose reaction to H_2SO_4 and IKI (×1250). (C and D) Later stages of the reaction shown in (B) (×1250).

showed no evidence of solution of the particles and the viscosities produced were but slightly above those of the cuprammonium solution itself (Table I). The behavior of the particles prepared through prolonged extraction with ammonium oxalate was of special interest in this group of viscosity determinations. Other techniques such as staining reactions, the degree of brightness in polarized light, etc. had shown previously that the cementing material was incompletely removed from the surfaces of the particles with this reagent. It is shown in Table I that the viscosity measurements of these particles were significantly higher than the viscosities of the samples extracted with other solvents.

TABLE II

EFFECT OF LENGTH OF TIME OF EXTRACTION OF COTTON FIBERS UPON CUPRAMMONIUM VISCOSITIES; TEMPERATURE 4° C.

Extraction medium	Length of treatment at 75° C.	G. per 100 cc.	Viscosity (time)
Cuprammonium solution	—	—	3 min. 34 sec.
Untreated	—	0.25	102 " 50 "
0.5% $(NH_4)_2C_2O_4 \cdot H_2O$	10 min.	0.25	50 " 44 "
"	1 hr.	0.25	28 " 16 "
"	4 hrs.	0.25	26 " 47 "
2% $(NH_4)_2C_2O_4 \cdot H_2O$	10 min.	0.25	33 " 54 "
"	4 hrs.	0.25	23 " 11 "
"	24 hrs.	0.25	23 " 2 "
1% KOH	10 min.	0.25	45 " 43 "
"	1 hr.	0.25	35 " 6 "
"	4 hrs.	0.25	25 " 38 "
2% HCl	10 min.	0.25	14 " 18 "
"	1 hr.	0.25	8 " 19 "
"	2 hrs.	0.25	6 " 57 "
"	4 hrs.	0.25	5 " 50 "
"	24 hrs.	0.25	5 " 24 "
2% citric acid	10 min.	0.25	35 " 32 "
" "	1 hr.	0.25	31 " 11 "
" "	2 hrs.	0.25	22 " 29 "
" "	4 hrs.	0.25	16 " 36 "
" "	24 hrs.	0.25	8 " 40 "

The next series of experiments dealt with viscosity measurements of fiber samples which had been extracted for different lengths of time in different mild extracting agents. The data shown in Table II are typical of those which were obtained. As the length of time of extraction increased, the curve for degree of viscosity descended, regardless of the extracting agent. These results are very similar to those obtained by Gibson (18) for cotton fibers treated for different lengths of time at different temperatures and different degrees of pressure with 2 to 4 per cent NaOH. He found,

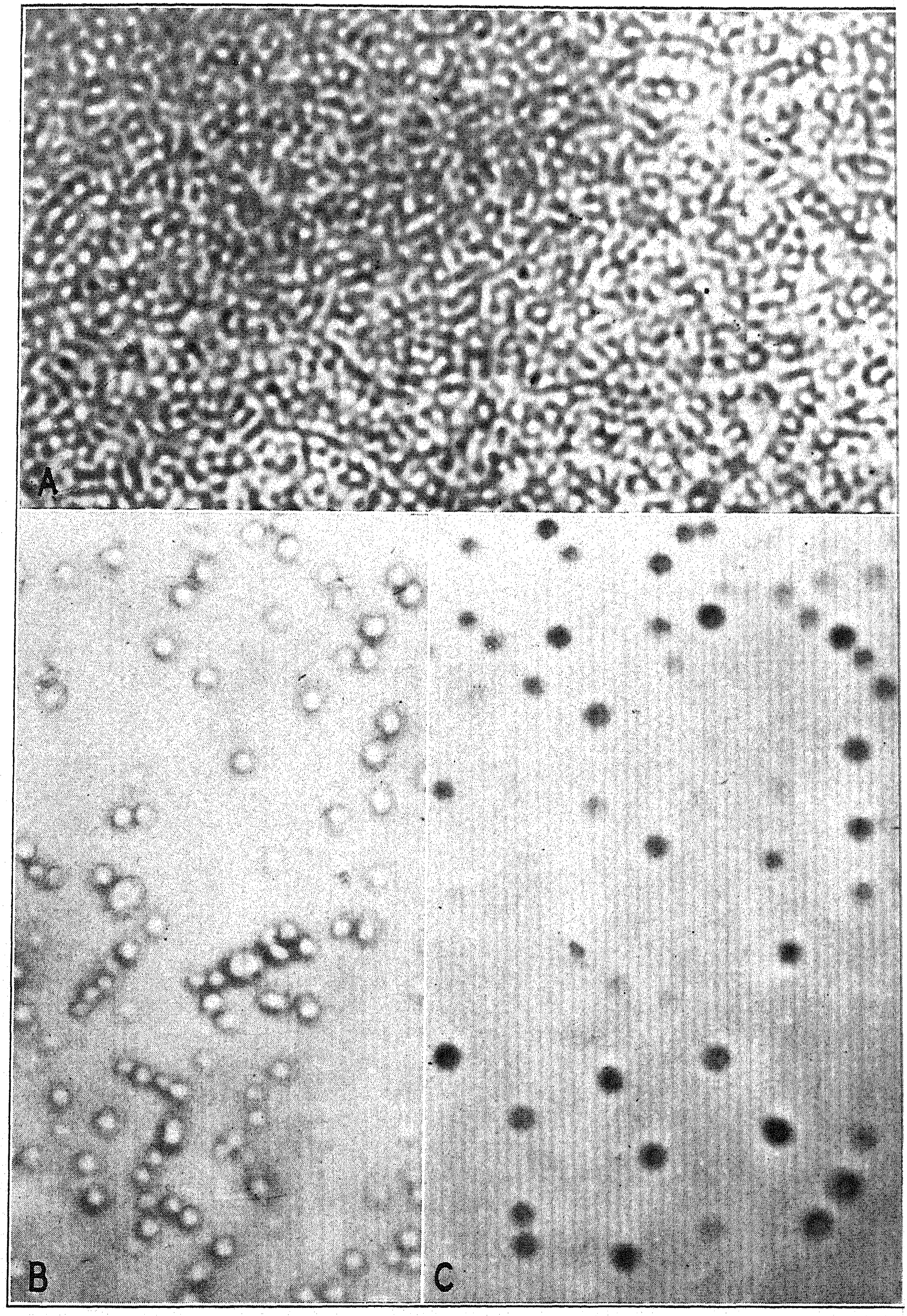

FIGURE 5. (A) Celulose particles from purified cotton linters in cuprammonium hydroxide (×1640). (B) Cellulose particles shown in (A) were not dissolved by the cuprammonium hydroxide but swell quickly in strong sulphuric acid (×1640). (C) When iodine is added to the mount they quickly color blue, the reaction characteristic of cellulose (×1640).

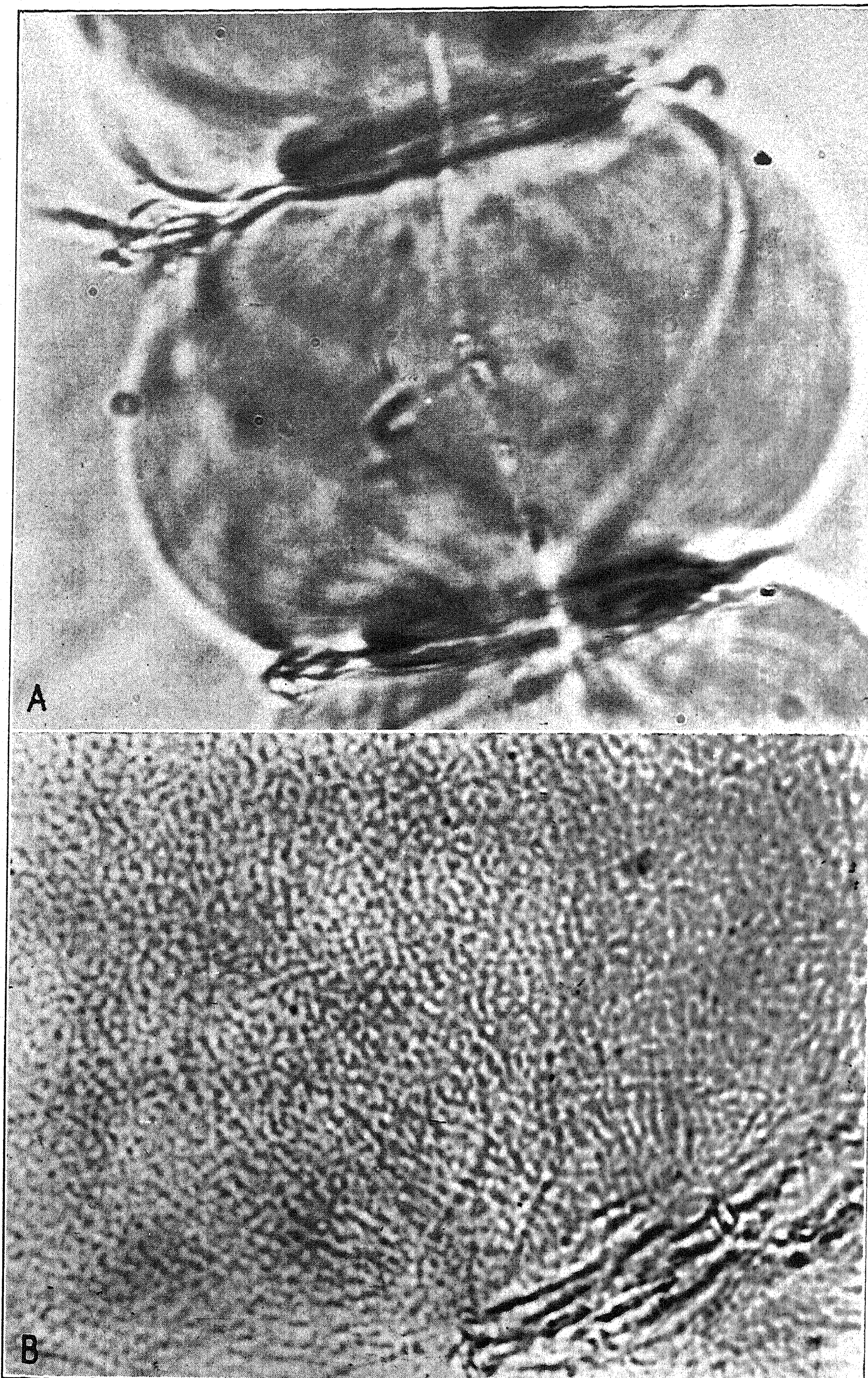

FIGURE 6. (For description see legend on opposite page.)

as did we, that the viscosity falls rapidly at first and then slackens. He also pointed out a relation between the degree to which the viscosity was lowered and the concentration of the solvent. This is also shown from our data (Table II) in a comparison of the effect of 0.5 and 2 per cent $(NH_4)_2C_2O_4.H_2O$. Another observation of Gibson's which we have corroborated with many different types of solutions during the past three years is that, as the proportion of extracting medium to fiber mass is increased, the viscosity of the residue is reduced. We have found, in this connection, that the rate at which a cell membrane can be disintegrated is dependent upon the nature of the solvent for the cementing material; the pH of the solution; the temperature of extraction; the degree of agitation; within certain limits, the frequency with which extractions are repeated; and, not least in importance, the quantity of the liquid in proportion to the fiber mass.

Gibson's interpretation of the fundamental changes in the fiber membranes associated with the decrease in their viscosity-producing power is of interest (18, p. 484): "The work has scarcely progressed far enough for wide generalizations to be made, but it seems clear that certain treatments can be applied to cellulose which alter its physical properties profoundly, but produce no decided change in its chemical properties. This lends support to the view that the production of cellulose in the plant is a process of polymerization, the older layers, presumably, being more highly polymerized than the new growth; the process of boiling with sodium hydroxide may effect the reverse change and depolymerize the cellulose."

The knowledge that the cellulose component of the membrane is in the form of diminutive particles surrounded by non-cellulosic cementing material (15) along with the observation that the cellulose particles do not dissolve in cuprammonium hydroxide to produce the viscosities commonly attributed to them, led us to search for an explanation of the lowering of viscosity with mild reagents other than that of depolymerization of the cellulose suggested by Gibson. The possibility that the cementing material (or some fraction of it) was of primary importance in the production of viscosities in the mixtures of cell membranes and cuprammonium hydroxide had been definitely suggested by our experimental data. From this point of view, the reduction of viscosity through treatment of the fiber mass with mild reagents could be interpreted as the result of a direct effect upon the viscosity-producing power of the cementing material.

FIGURE 6. (A) An early stage of swelling of a single cotton fiber in cuprammonium hydroxide. The rapidly enlarging inner layers of the membrane break through the outer layer, which does not swell in the reagent, forming a series of globe-like structures (×1250). (B) A later stage of treatment of the same fiber with cuprammonium hydroxide. The swollen cementing material which obscured the cellulose particles in (A) has been carried away and the particles, in random arrangement, are clearly visible throughout the field (×1250).

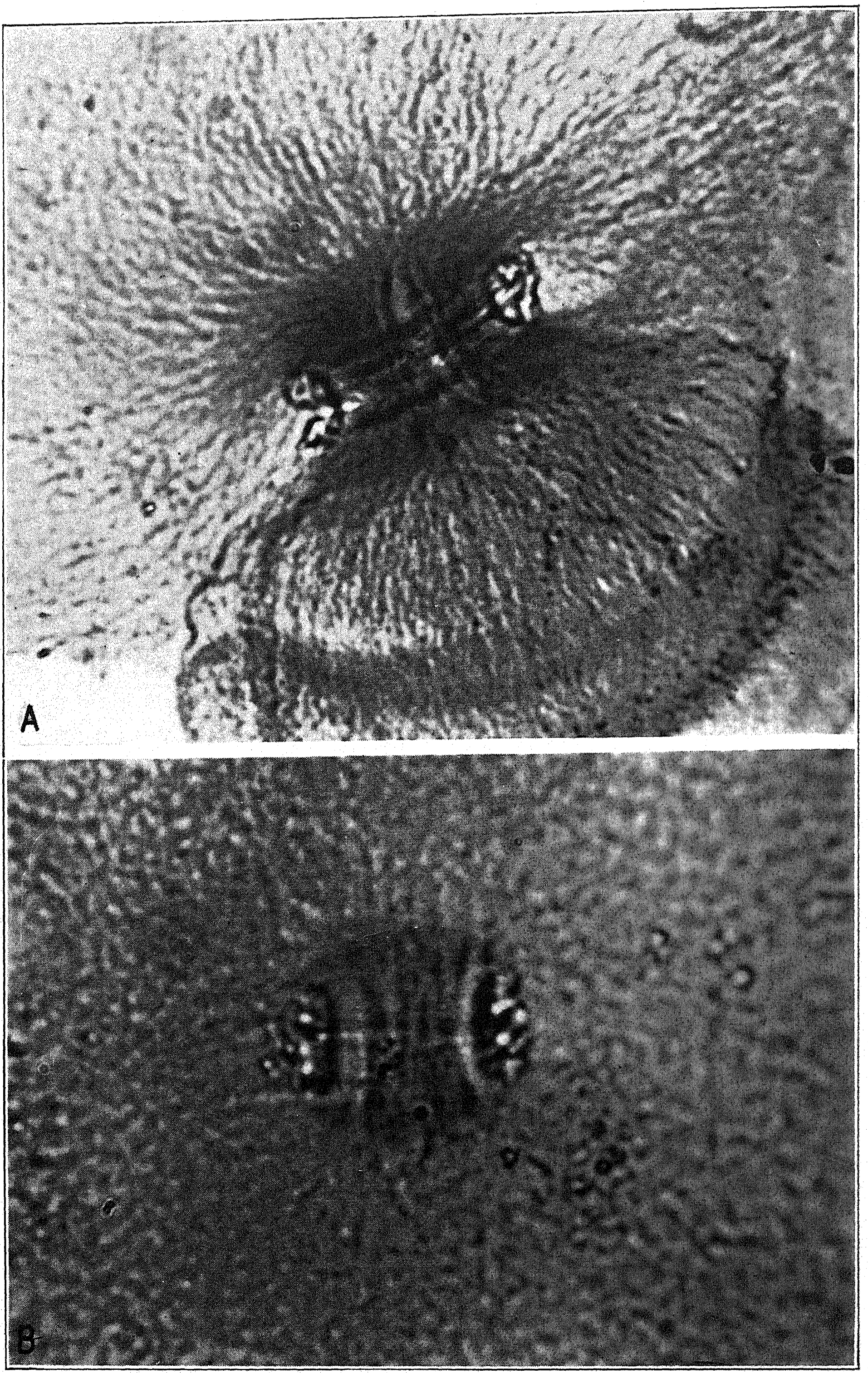

FIGURE 7. (For description see legend on opposite page.)

It was not difficult to believe that such extracting agents as 1 to 2 per cent KOH, 2 per cent HCl, 0.5 to 2 per cent $(NH_4)_2C_2O_4.H_2O$, etc. would drastically alter the colloidal properties of the cementing material. We had been using these reagents for several years in our work dealing with the extraction of various fractions of the cementing material and had learned that only the most careful control of the experimental conditions would avoid complete destruction of their colloidal properties. The question was whether or not any one of the extracted and purified fractions of this cementing material would produce a measurable increase of viscosity in cuprammonium hydroxide. The one fraction of the cementing material of the cotton fiber which we had been able to separate and identify in sufficient quantities for such a study was the component which is extracted with 0.5 per cent ammonium oxalate. Upon the basis of uronic acid anhydride determinations according to the method of Dickson, Otterson, and Link (10)[3] this extract was identified as pectic material (Table III). During the extraction and purification of the material, care was taken to maintain its colloidal properties by controlling the temperature, the pH of the dispersions, and by avoiding excessive drying. It is well known, however, that every necessary manipulation of such a substance produces

TABLE III

URONIC ACID ANHYDRIDE DETERMINATIONS

Source of material	Extraction medium	% CO_2	% Uronic acid anhydride	% Pectin
Cotton	2% Ammonium oxalate	14.034	56.136	79.43
"	" " "	14.036	56.144	79.44
Lemon	" " "	16.478	65.92	93.27
Apple	Commercial	15.674	62.696	88.71
Citrus	"	16.633	66.532	94.14

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[3] This method is based upon the original observation of Lefèvre and Tollens that when glucuronic acid is heated with 12 per cent HCl (sp. gr. 1.06) it is decomposed according to the following equation:

$$C_6H_{10}O_7 = C_5H_4O_2 + CO_2 + 3H_2O$$

From extensive studies of plant materials, Dickson, Otterson, and Link found that a free glucuronic acid is present in the cell, that a polymerized glucuronic acid comprises part of the pectinaceous substances of the cell and cell wall and that glucuronic acid is also intimately associated with the cellulose of the cell wall. The percentage of CO_2 obtained from a given sample multiplied by 4 gives the percentage of uronic acid anhydride. The percentage of carbon dioxide multiplied by 5.66 gives the percentage of pectic material. The uronic acid anhydride values of highly purified pectin vary from 70 to 73 per cent.

FIGURE 7. (A) Cellulose particles, in a single fiber, which have not been swollen by the cuprammonium hydroxide quickly begin to swell and turn blue with sulphuric acid and iodine (×910). (B) A late stage of swelling with sulphuric acid alone (×910).

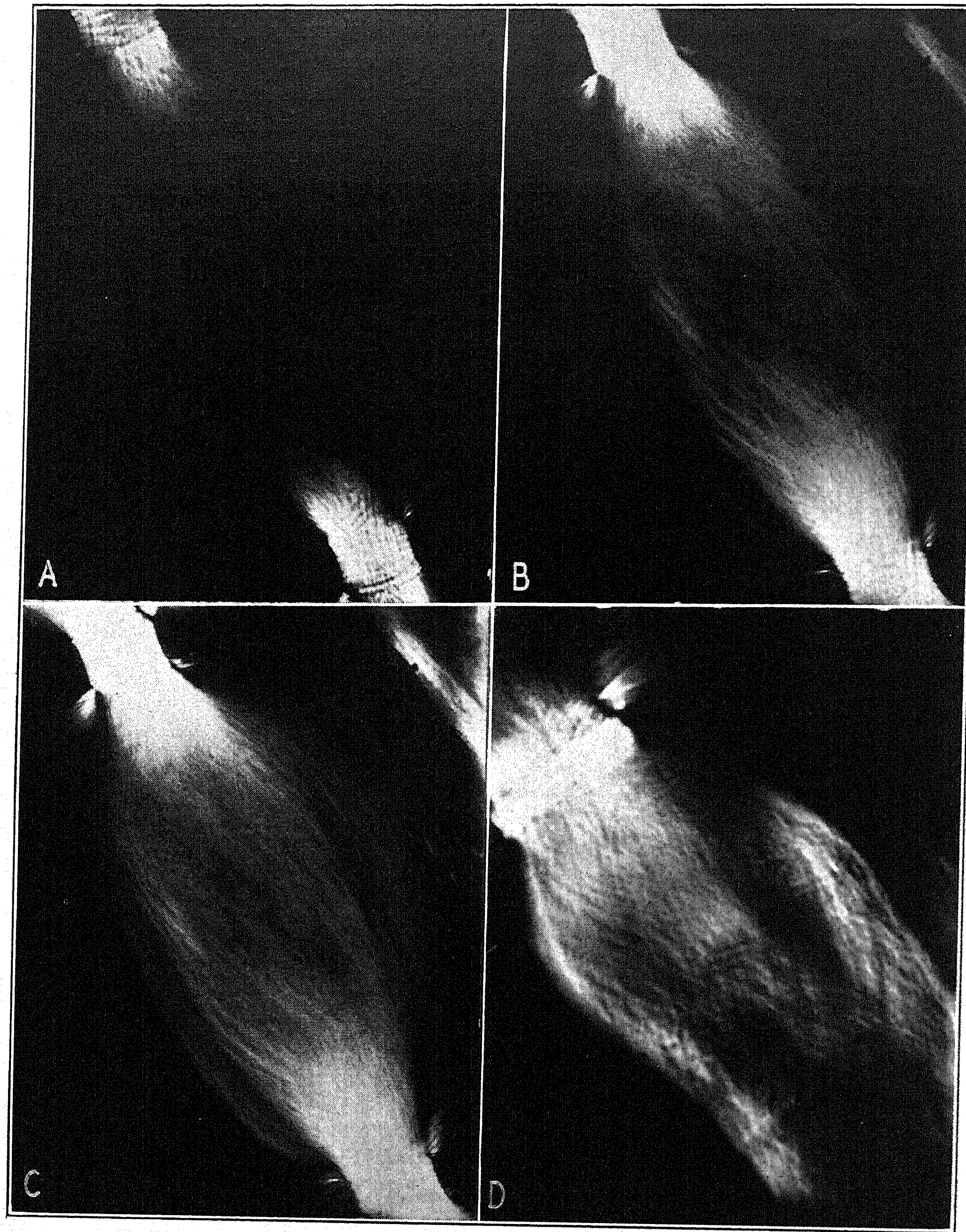

FIGURE 8. A single cotton fiber in polarized light. (A) In the early stage of treatment with cuprammonium hydroxide, the swollen cementing material obscures the cellulose where the enlargement is unrestrained by the constricting bands of the cuticle (×205). (B, C, D) Continued treatment with cuprammonium hydroxide washes away the cementing material and the double refractive cellulose again becomes visible (B and C, ×205, D, ×430).

changes in its colloidal consistency which, although not entirely irreversible, are certainly not completely reversible. It must be emphasized, therefore, that in studying directly the viscosity-producing power of this extracted pectic substance, we were using only one of the possible viscosity-producing components of the cementing material and that its original viscosity-producing power had been markedly reduced by extraction and repeated purification.

When 0.625 g. of this powdered pectic substance was added to 100 cc. of the A.C.S. standard solution of cuprammonium hydroxide, the viscosity of the cuprammonium solution was increased from 3 min., 43 sec., to 11 min., 24 sec. The addition of 1.25 g. to 100 cc. of the solution increased the viscosity to 26 min., 10 sec. Table IV shows the result of adding increasingly larger amounts of pectic material to the point when over 10 hours were required for the mixture to flow through the viscosity tube. Any quantity of this pectic extract over 5 g. per 100 cc. produced a gel so stiff that it would not flow (Fig. 1 B).

TABLE IV

VISCOSITIES OF PECTIC MATERIAL IN CUPRAMMONIUM HYDROXIDE*; TEMPERATURE 4° C.

Source of pectic material	Extraction medium	G. per 100 cc.	Viscosity (time)
Cotton fibers	0.5% ammonium oxalate	0.625	11 min. 24 sec.
“ “	“ “ “	1.25	26 “ 10 “
“ “	“ “ “	1.87	49 “
“ “	“ “ “	2.5	135 “
“ “	“ “ “	3.75	283 “ 21 “
“ “	“ “ “	5.0	682 “ 13 “
“ “	5% citric acid	5.0	135 “
Lemon albedo	Water extract	2.0	30 “ 51 “
Commercial pectin (Eimer and Amend)		2.5	50 “ 54 “

* The viscosity of the cuprammonium solution itself was 3 min., 43 sec.

Similar results were obtained with the colloidal substance extracted from cotton with 5 per cent citric acid, although a 5 g. sample of this material produced a much less viscous mixture than a 5 g. sample of the ammonium oxalate-extracted pectin. The viscosity-producing power of a 2 g. sample of water-extracted lemon pectin and of a 5 g. sample of commercial pectin is also shown for general comparison (Table IV). The measured viscosity of the commercial sample is approximately one-third of that of a comparable sample of cotton pectin. Uronic acid analyses of these particular samples were not made previous to the study of their behavior in solutions of cuprammonium hydroxide. These miscellaneous examples are cited in order to further emphasize the need for more knowledge of the viscosity-producing power of the non-cellulosic cell membrane constituents under a wide range of experimental conditions.

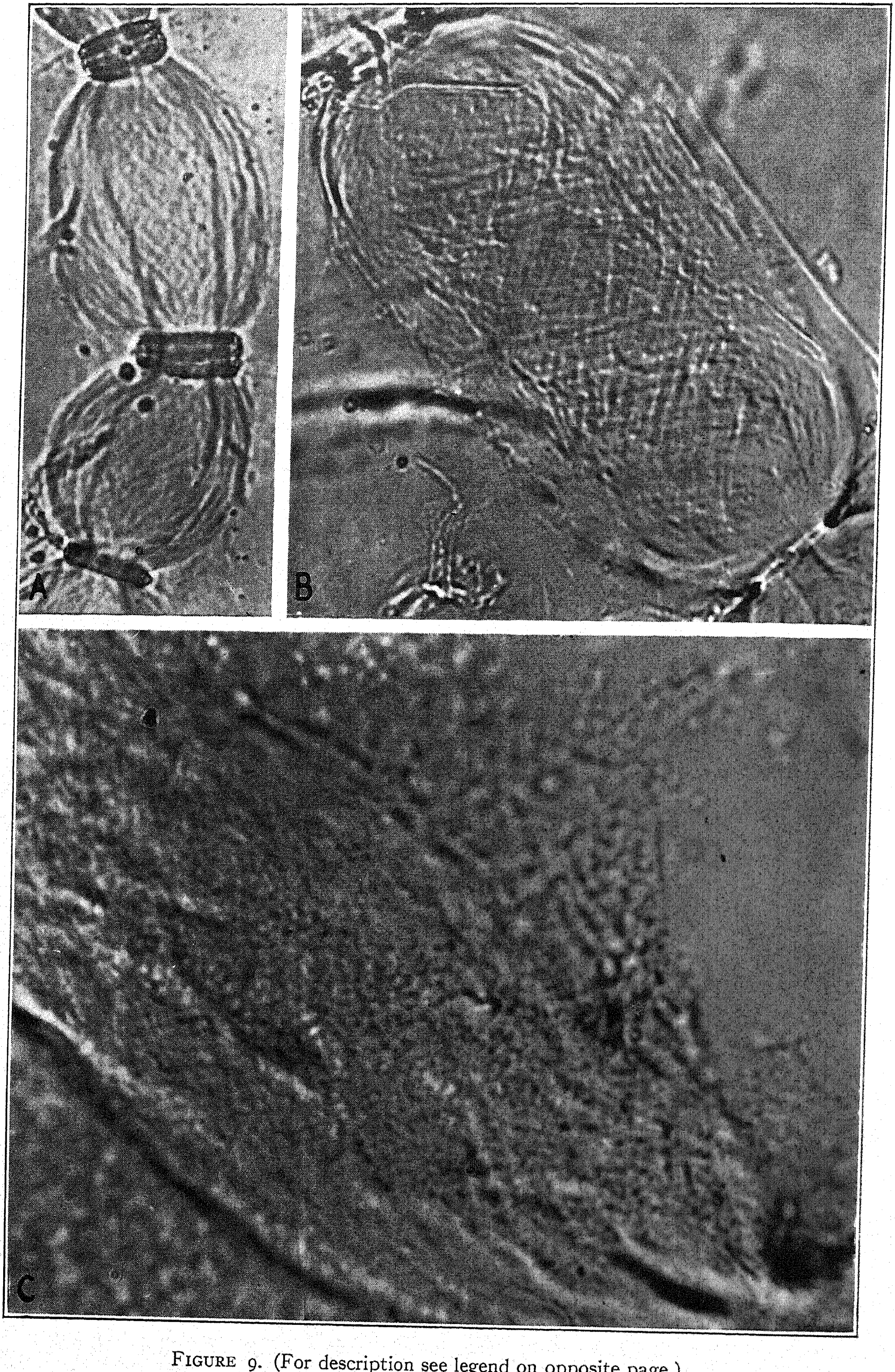

FIGURE 9. (For description see legend on opposite page.)

The mixtures of pectic material and cuprammonium hydroxide were distinguished by their sparkling clarity, and their greatly reduced tendency to "separate" as compared with the mixtures containing either raw cotton or both extracted pectic material and separated cellulose particles.

The nature of the combination of this pectic substance, extracted by ammonium oxalate, with cuprammonium hydroxide is not known. We learned, however, that this one fraction of the cementing material, even after marked alteration of its original colloidal properties, can still produce viscosities in the cuprammonium solution to the point of formation of a stiff gel. Since the cellulose particles do not dissolve to produce the viscosities commonly attributed to the cellulose component of the membrane, we may assume that their presence in a mixture of cuprammonium hydroxide and cementing material contributes to the viscosity only in the sense that any inert material in a state of dispersion would bring about an apparent increase in the viscosity of an already viscous mixture.

In the discussion of these results following their verbal presentation (11) the question arose as to whether or not the behavior of our fiber material was comparable to the behavior of the purified cotton used commercially. From the viewpoint of many cellulose chemists we were dealing with "plant cellulose" while their experimental material in the form of purified cotton or wood pulp was referred to as "chemical cellulose." The "high viscosity" cellulose of commerce, consisting, as was believed, of soluble cellulose in a purified state, had the possibility of producing, in comparable concentrations, viscosities that were higher than any which we had obtained with raw cotton. In the interest of learning the viscosity-producing power of these purified commercial samples as compared with our own, under the same experimental conditions, we obtained, through the courtesy of Hercules Powder Company, three carefully prepared samples of Hercules linters which were classified as follows: sample No. 2880, high viscosity; No. 2879, medium viscosity; No. 2881, low viscosity. The results of viscosity measurements of these samples are shown in Table V. The value obtained for the "high viscosity" sample was less than half as great as that of our untreated Super Seven cotton; the value for the "medium viscosity" was approximately one-tenth of this amount; and the value for the "low viscosity" sample was but slightly higher than that of the cuprammonium solution itself. These samples fell well within the range of viscosities of our own experimental material and were, from our viewpoint, merely cotton fibers from which the cementing material

FIGURE 9. Single fibers of "purified" cotton in cuprammonium hydroxide. (A, B) Early stages of swelling show detail in fiber structure due to the previous removal of cementing material during the process of purification (×455). (C) Fiber disintegration is rapid as compared with untreated cotton and the cellulose particles, less restrained by the colloidal matrix of low viscosity, are floated along in the current of the reagent (×1090).

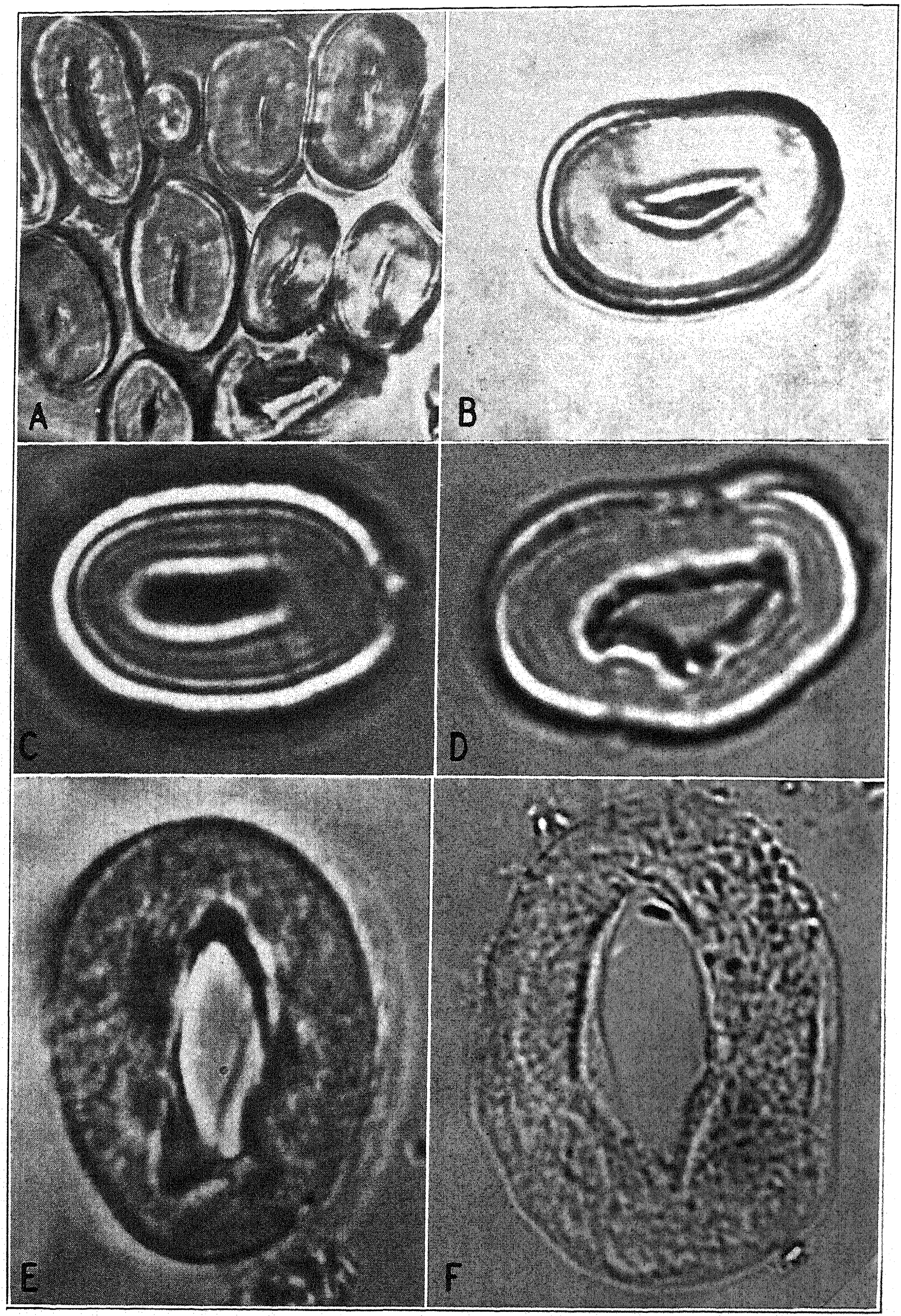

FIGURE 10. (For description see legend on opposite page.)

had suffered partial extraction and alteration of its colloidal properties through the previous "purifications" to which the fibers had been subjected. Treatment of some of the high viscosity linters for 72 hours at 75° C. with distilled water and no agitation reduced their viscosity-producing power in cuprammonium hydroxide from 43 min., 15 sec., to 25 min., 15 sec.

TABLE V

VISCOSITIES OF PURIFIED COTTON LINTERS IN CUPRAMMONIUM HYDROXIDE; TEMPERATURE 4° C.

Sample	G. per 100 cc. cuprammonium	Viscosity (time)
Cuprammonium	—	4 min. 6 sec.
Hercules linters #2881 "low viscosity"	0.25	7 " 30 "
Hercules linters #2879 "medium viscosity"	0.25	13 " 3 "
Hercules linters #2880 "high viscosity"	0.25	43 " 52 "
Hercules linters #2880 extracted at 75° C. for 72 hrs. with distilled H_2O	0.25	25 " 15 "
Untreated cotton, Super Seven variety	0.25	102 " 50 "
Hercules linters #2881 "low viscosity"	1.0	32 " 37 "

Microscopic examination of the Hercules linters by means of all of the variations in this technique available to us corroborated this interpretation. Fiber disintegration takes place to a marked degree in the course of preparation of the natural fibers for commercial use. The fibril and particle structure of these purified linters is clearly visible after a comparatively brief treatment with the reagents which we use for fiber disintegration (Fig. 4 A). When these fiber fragments were dissected apart and treated with strong sulphuric acid and iodine, the particulate structure of the fibrils and the cellulose nature of the particles became apparent (Fig. 4 B). Later stages of the reaction of these particles to sulphuric acid and iodine are shown in Figure 4 C and D. The latter shows the particles on the border line of complete physical dissolution in the course of their hydrolysis to glucose. These observations of *cellulose degradation* in purified linters by treatment with sulphuric acid revealed no differences from the same phenomenon as it is observed in untreated cotton. The real differences

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FIGURE 10. (A) Cross sections of cotton fibers, 2–3μ in thickness, show marked variations in size and shape (×835). (B) An early stage of swelling in cuprammonium hydroxide (×835). (C) A later stage of swelling of a section across a region where the fibrils run parallel to the fiber axis shows the fiber lamellae (×1360). (D) Swelling of a section across a region where fibrils are at an angle with the fiber axis shows uneven swelling of the lamellae (×1360). (E) In a later stage the rapidly swelling cementing material distorts the lamellae and obscures the particles (×1270). (F) Continued washing with cuprammonium hydroxide carries away the swollen cementing material and the particles are more clearly visible (×1270).

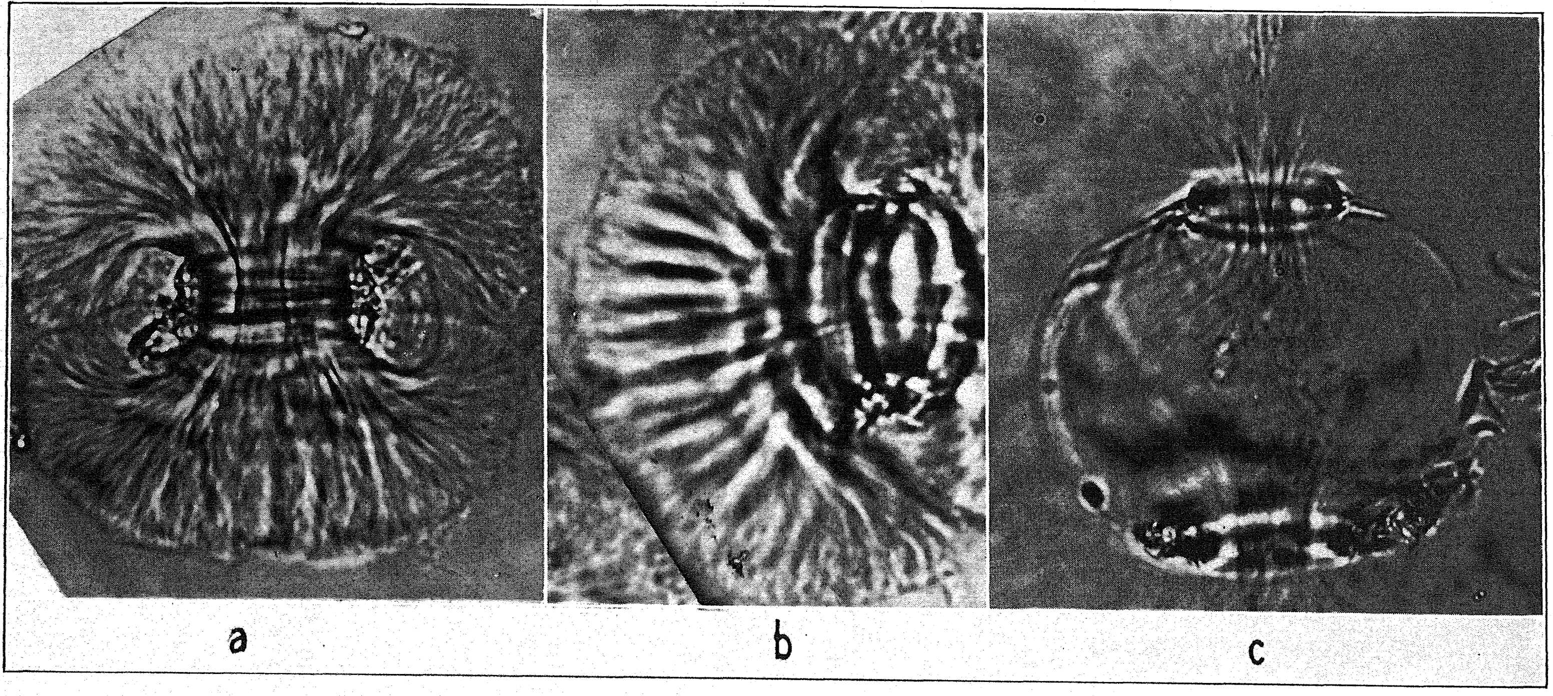

FIGURE 11. Cross-sections of cotton fibers 10–15 microns in thickness swell in cuprammonium hydroxide in the three ways shown in these illustrations. (a) The cuticle which does not swell in the reagent forms a band around the center of the section which continues to swell in the form of a sheaf of wheat. (b) The cuticle constricts one end of the cross-section and the fibrils radiate out from the base of this band in all directions. (c) The cuticle breaks in the center of the section and constricts both ends, the inner lamellae swelling out in a lantern-like fashion. End views of (b) and (c) show the ring of cuticle in the center of the respective structures.

observed in the inaccurately called "chemical cellulose" and "plant cellulose" are in their comparative rates of *fiber disintegration* and in their comparative viscosity-producing powers in solutions of cuprammonium hydroxide, both of which are concerned with the chemical and physicochemical states of the *cementing material*. Cellulose degradation on the other hand is associated specifically with changes in the cellulose particles, and fiber disintegration with changes in the cementing material in both native and purified cotton fibers.

A photomicrograph of cellulose particles from Hercules linters in the standard solution of cuprammonium hydroxide (Fig. 5 A) is strictly comparable to a similar preparation of particles from raw cotton in this reagent shown in Figure 3 B. They likewise react, after dispersion, to sulphuric acid and iodine, in a manner characteristic of cellulose particles in both developing and mature cotton fibers (Fig. 5 B and C).

The swelling of the single cotton fiber in solutions of cuprammonium hydroxide strong enough to produce fiber disintegration is one of its most familiar and most frequently illustrated reactions. The characteristic globe-like swellings along the fiber axis have been observed and explained in various manners for the past eighty years with no important additions to our knowledge of the phenomenon beyond that contributed by Schweizer (33) and by Cramer (9). They recognized the fact that the swelling fiber had the appearance of a strand of beads because of the comparative insolubility of the outer sheath of the fiber in cuprammonium hydroxide. This outer limiting membrane is ruptured into a series of band-like constrictions by the expanding inner lamellae of the fiber wall.

In ordinary light the microscopic appearance of the globe-like swelling of an unpurified cotton fiber, is that of a structureless gel (Fig. 6 A). This has been interpreted as evidence of the very rapid "solution of the cellulose lamellae" in the reagent. The process is rapid and soon reaches a point where the entire field appears under ordinary optical conditions, as a structureless matrix through which are scattered the isolated cuticular rings. Only careful microscopic technique is adequate to assist in discerning what is really taking place during the various stages of this process. Well made, properly adjusted lenses, critical illumination, the thinnest of slides and cover glasses of clear, white glass, and well controlled application of the reagent reveal the differential reaction of the cellulose particles and the cementing material which surrounds them.

The single fiber is first mounted in distilled water. This medium is very gradually replaced with the cuprammonium solution by drawing it under the cover glass with very narrow (2 mm.) strips of filter paper. In this way not only the rate but also the direction of flow may be altered so that, depending upon the position of the fiber axis with respect to the direction of the current, the entire reaction is under an unusual degree of control.

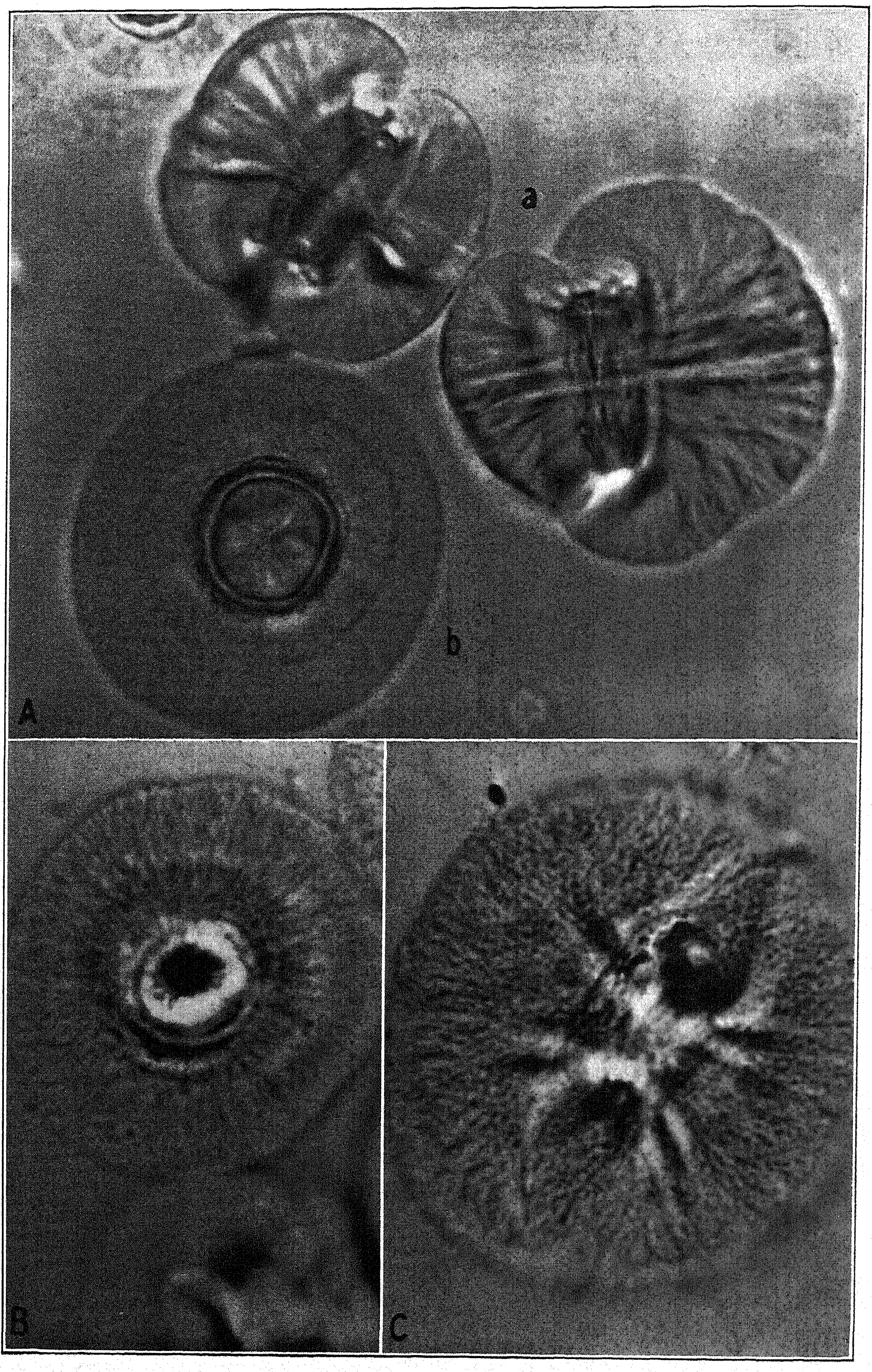

FIGURE 12. (For description see legend on opposite page.)

The fiber begins to swell. The cuticle ruptures in many positions along, and at right angles to the fiber axis. This insoluble outer layer is soon transformed by the rapid enlargement of the inner layers of the wall into an intermittent series of crushed bands. The comparatively immense blue globe-like swellings between these constrictions appear to be structureless (Fig. 6 A). At this point carelessness or haste in supplying the reagent can alter entirely one's conception of the nature of the reaction. A steady flow of fresh cuprammonium solution must be maintained to prevent crystallization of the highly concentrated copper salt. If it is applied in too large drops and drawn through too rapidly, the swelling proceeds with an almost explosive result. The usual procedure at this critical stage of the swelling is to replace quickly the cuprammonium hydroxide with ammonia alone and the "dissolved" cellulose is "regenerated" in a granular disorganized state. If, however, at this point of swelling (Fig. 6 A) we continue to supply, with the greatest care, the fresh cuprammonium solution instead of ammonia to the mount, a microscopic observation is obtained which completely alters our previous conception of the nature of the reaction. The globe-like structure—usually only one in the field at high magnification—very slowly increases in diameter. As the solution passes over it, the expected gradual disappearance of all traces of the fiber from the field does not occur. Out of the apparently structureless blue mass, uniform-sized granules begin to appear as though a veil which had been covering them were being slowly carried away (Fig. 6 B, a late stage of swelling of Fig. 6 A). These granules are the cellulose particles which had not dissolved in the cuprammonium hydroxide but which had been obscured to the point of invisibility by the swollen matrix of colloidal cementing material which surrounded them. The slow movement of the fresh cuprammonium hydroxide through the mount had gradually removed a part of the swollen colloidal substance without washing away the denser particles. The addition of ammonia alone produces a similar effect but has been previously interpreted as "recrystallization" (19) or as "regeneration." Although neither the descriptions nor the illustrations enable us to understand the nature of the tissue reactions obtained by Gilson, it now seems probable that, in the cells which he examined, the cellulose particles were merely temporarily obscured by the swollen colloidal material surrounding them. Unaltered in shape and size by the cuprammonium hydroxide, the cellulose particles, in our single fiber reactions, show the characteristic behavior in sulphuric acid and iodine, the early and late stages of which are shown in Figure 7 A and B.

FIGURE 12. (A) Early stages of swelling of *thick cross-sections* (10–15μ) of cotton fibers in cuprammonium hydroxide. Two examples of type (a) and one example of type (b) are shown (×950). (B) A late stage of swelling of type (b) (×950). (C) A late stage of swelling of type (a) (×950).

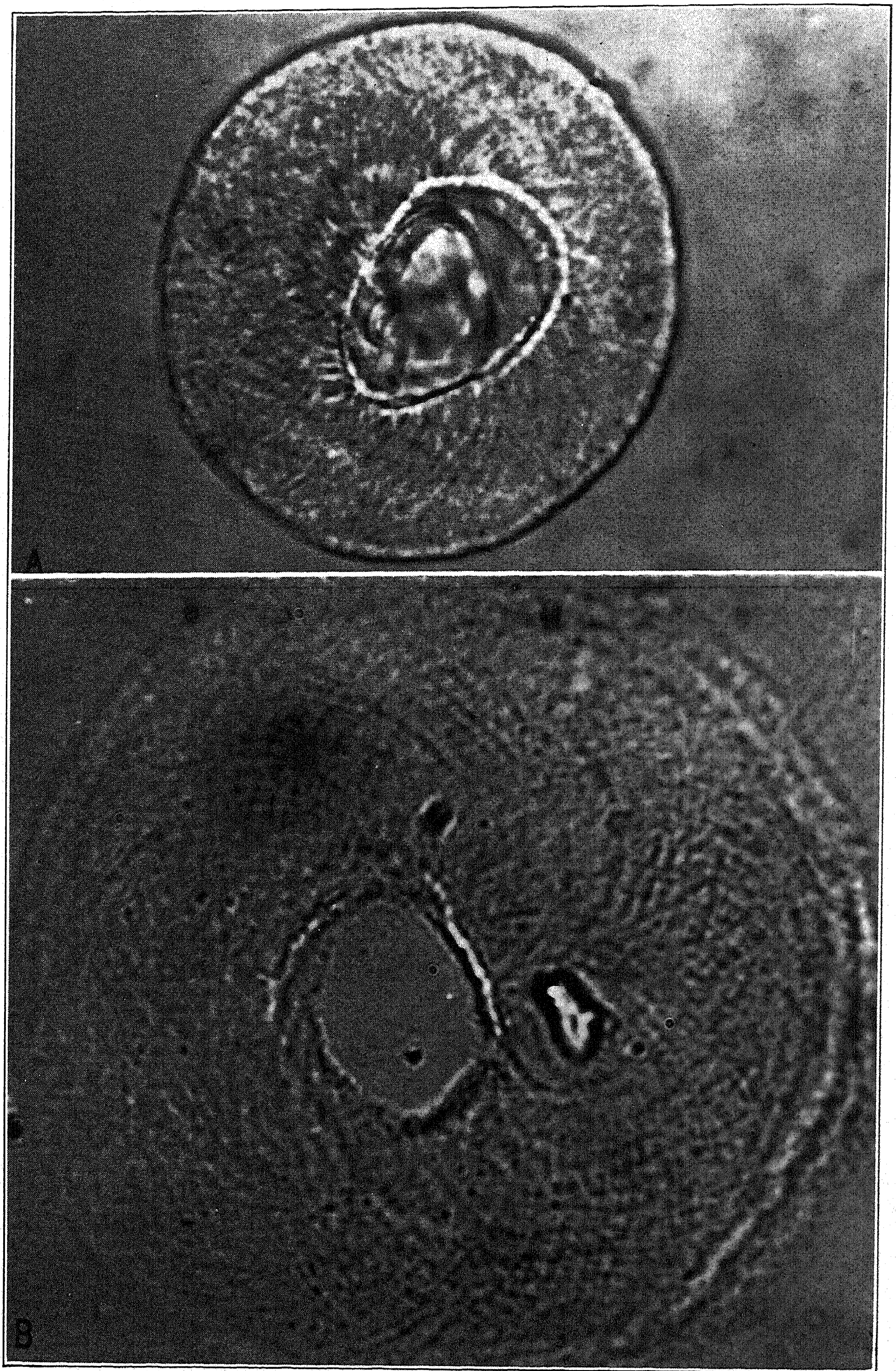

FIGURE 13. (For description see legend on opposite page.)

It is of special interest to follow the swelling reaction of a single fiber in polarized light under the same experimental conditions. Both Schweizer and Cramer had noticed that the fibers in the region of the constricting bands are strongly doubly refractive and that the globes are non-doubly refractive. Figure 8 A illustrates this point. The non-double refraction of the swollen area has been interpreted in the sense of the more rapid solution of the doubly refractive cellulose in this region. Continued washing with cuprammonium hydroxide shows clearly, however, that the double refraction of the cellulose in the globe has been merely obscured by the swollen cementing material. As this swollen material is slowly carried away, the double refraction of the cellulose again becomes evident (Fig. 8 B, C, and D).

Single fibers of purified cotton linters react to cuprammonium hydroxide in a manner which we would readily expect from our knowledge of the alteration of the cementing material during the process of purification. Figure 9 A, B, and C illustrates the facts that the swollen areas are much less robust and that the intricate fibril and particle structure is less obscure in all stages of swelling and disintegration of a purified fiber. This behavior of purified cotton linters is a confirmation by microscopic technique of the information obtained from the same material by means of viscosity measurements.

Microscopic observations of cross-sections of cotton fibers in cuprammonium hydroxide equal, in interest, these single fiber studies. Our own work with cross-sections of cotton fibers[4] has extended over a period of several years. In the course of that time we have learned how to section mature fibers from a boll, not yet at the stage of natural opening, without any form of infiltration and imbedding; have found that only cross-sections 2–3 microns in thickness are suitable for studies of the lamellate structure; and have learned that uniform swelling throughout the section is dependent upon a straight cut, perpendicular to the fiber axis, across an area where the fibrils run parallel to the fiber axis—the so-called "position of extinction" in polarized light (13).

The number of lamellae in the variety of cotton which we use for experimental purposes (*Gossypium hirsutum* L. Super Seven, Strain 4) varies from about 4 to 15. A cross-section whose membrane width is 13

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[4] A comprehensive study of the cross-sectional structure of cotton fibers in both intermittent and continuous illumination will be published by Dr. Florence Barrows in a forthcoming issue of the Contributions from Boyce Thompson Institute.

FIGURE 13. (A) An early stage of swelling of a thick cross-section of type (c). Cross-spiral arrangement of fibrils upon surface of the lantern-like structure is visible (×950). (B) A late stage of swelling of type (c). No "growth rings" are visible at any stage of swelling without pressure (×1250); see Figure 14 for pressure artifacts.

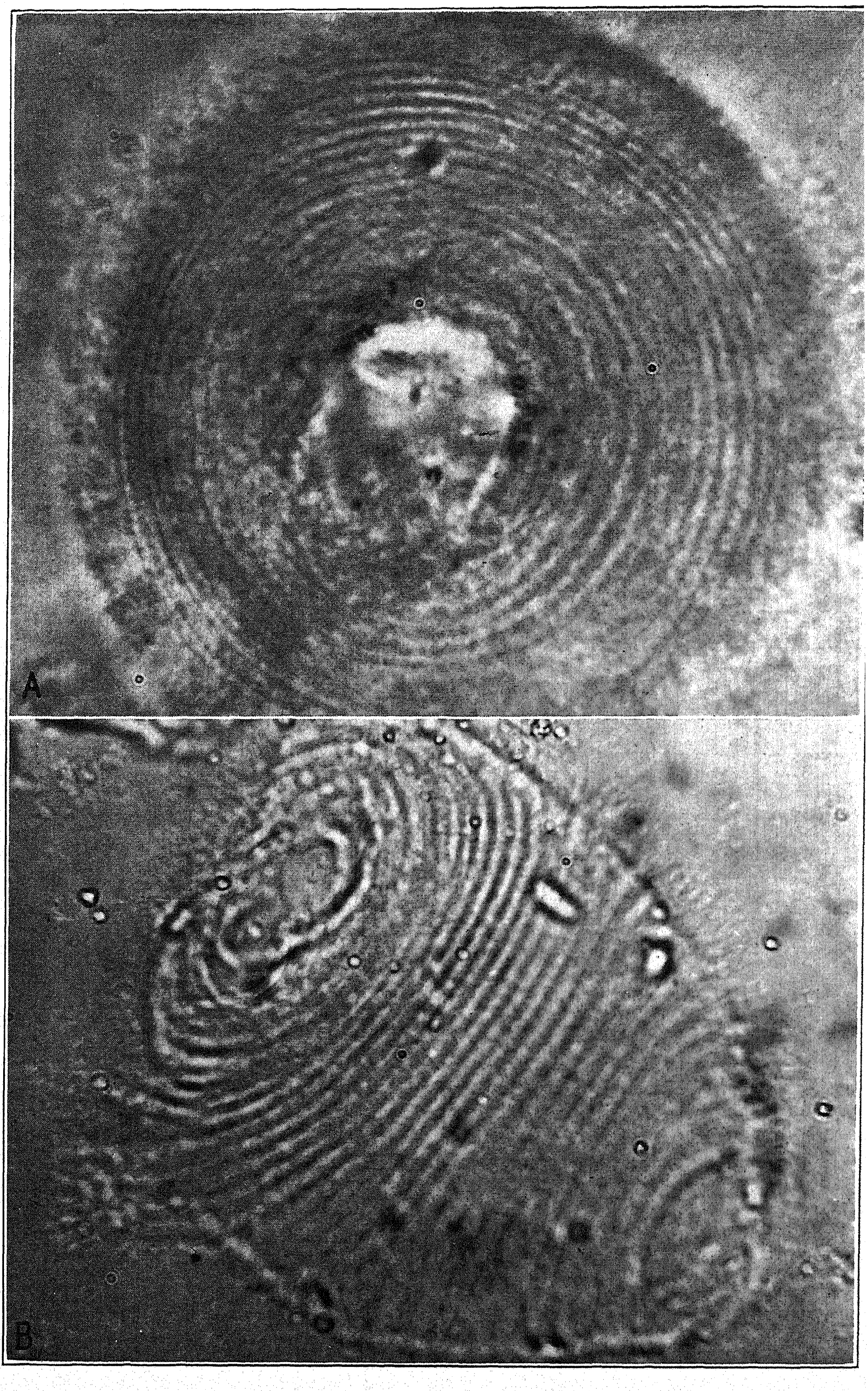

FIGURE 14. (For description see legend on opposite page.)

microns contains, for instance, 10 to 12 lamellae. All measurements, counts, and direct observations show that each lamella is made up of a single layer of cellulose particles. Thin layers of cementing material surround each particle and alternate with each lamella. The lamellae containing the particles are doubly refractive and only those containing the cementing material alone are non-doubly refractive, thus producing in the swollen state the familiar concentric, alternating light and dark areas in polarized light.

Our microscopic results with cross-sections of the fibers swollen in cuprammonium hydroxide may be summarized with the illustrations in Figure 10. The thin cross-sections are delicate and must be swollen with the greatest care. As swelling proceeds the shape of an oval section is retained and the lumen enlarges as the section enlarges (Fig. 10 A and B). The outer limiting membrane, which does not swell in cuprammonium hydroxide, does not interfere with the swelling behavior of such a thin cross-section. As swelling continues the lamellae become visible. In Figure 10 C the regularity in swelling is due to sectioning across a particular portion of the fiber in which the fibrils of the membrane run parallel to the fiber axis. Figure 10 D shows the type of distortion of the lamellae during swelling when the section is across a portion in which the fibrils are in a spiral position or at an angle with the fiber axis. The rapid swelling of the cementing material in such a thin cross-section quickly distorts the lamellate structure, and very nearly obscures the separated cellulose particles (Fig. 10 E). Longer continued washing with the cuprammonium hydroxide serves to carry away this swollen matrix, however, until the disarranged particles are clearly visible (Fig. 10 F).

This description of cross-sectional structure and swelling behavior has no points in common with the "growth rings" reported by Anderson and Kerr (4) and Bailey (5). They used sections 10 to 15 microns thick; ours were 2 to 3 microns in thickness. Soon after swelling in cuprammonium hydroxide begins, their sections turn inside out so that the outer part of the fiber occupies the former position of the lumen; we have observed no such phenomenon in the swelling of the thin cross-sections. The outline of their cross-sections in the final stages of swelling is shown to be circular and the diameter of the opening in the center of the section is very small in proportion to the diameter of the cross-section itself; our cross-sections retain their original outline during swelling and the lumen enlarges progressively as the swelling proceeds. In order either to stop the reaction with the cuprammonium solution or to assist in the reversion of

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FIGURE 14. (A) The effect of pressure upon the cover slip during the swelling of type (c) is to produce a series of concentric ridges with the central ring of cuticle as a point of origin (×1000). (B) When the lantern-like structure is turned upon its side, both rings of cuticle act as points of origin (×1000).

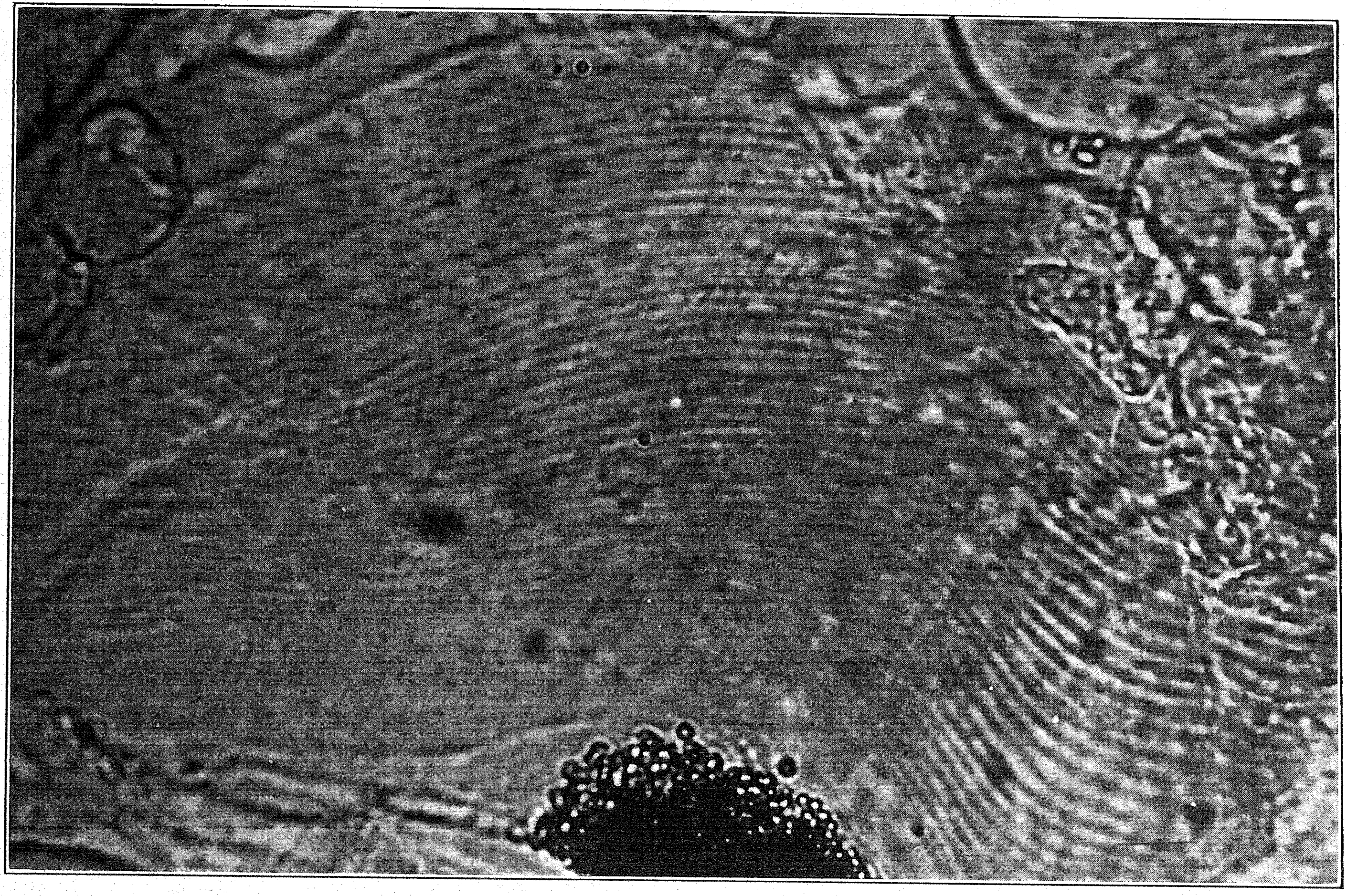

FIGURE 15. (For description see legend on opposite page.)

the "growth rings" they exert pressure upon the swollen section; the slightest pressure upon our cross-sections at any stage of swelling produces so much distortion that all of the original tissue structure is destroyed.

The preparation of cross-sections according to the procedures described by Kerr (21) has furnished an explanation of the discrepancies between their results and ours. In cross-sections 10 to 15 microns in thickness the cuticle plays an important role during swelling with cuprammonium hydroxide. It is observed to behave in three different ways (Fig. 11):

(a) The cuticle is rolled back to the center of the section and the swollen structure resembles, in outline, a sheaf of wheat. Early and late stages of swelling of this type are shown in Figure 12 A and C. The particle structure in the late stage is made clear again by washing away the cementing material with fresh cuprammonium hydroxide (Fig. 12 C).

(b) The cuticle constricts one end of the swelling section and the separating fibrils radiate out from the ring of cuticle as a center, when the structure is viewed from the top (Fig. 12 B). This explains the presence of the cuticle in the center of the Anderson and Kerr cross-section. Its location there is not due, however, to the reversal of the inner and outer portions of a cross-section.

(c) The cuticle splits in the center and a band of it constricts each end of the swelling fiber section (Fig. 11 c). This produces a lantern-like structure. If these sections begin to swell with their long axes parallel to the surface of the microscope slide, they quickly tip upon the curve of their swollen side, to a perpendicular position. This is the only motion observed during the swelling of the thick sections which could have been mistaken for a reversal of the inner and outer portions of a true cross-section.

Two or more of these three types of swelling may appear in a single field in the microscope (Fig. 12 A, a, b). Only rarely does one type alone occur. Type (c) is evidently the one selected by Anderson and Kerr to demonstrate the "growth rings" in the wall of the fiber. They show, however, only the final stage of swelling. When the intermediate stages are followed, the crossed fibrils of the surface of the fiber come clearly into view (Fig. 13 A). If swelling continues without pressure, the swollen structure is disintegrated without the appearance of "growth rings" in any part of it (Fig. 13 B). There is merely a progressive spreading apart of the fibrils and particles and finally the disappearance of all organized structure with only the cellulose particles in random arrangement in the field. If, however, after the swelling is well begun, pressure is exerted upon the cover glass of the mount, the entire swollen mass is thrown into a series of concentric

FIGURE 15. Gum tragacanth swollen with water. Pressure upon the cover glass produces the parallel ridges radiating out from a small mass of foreign material as a point of origin (×1040).

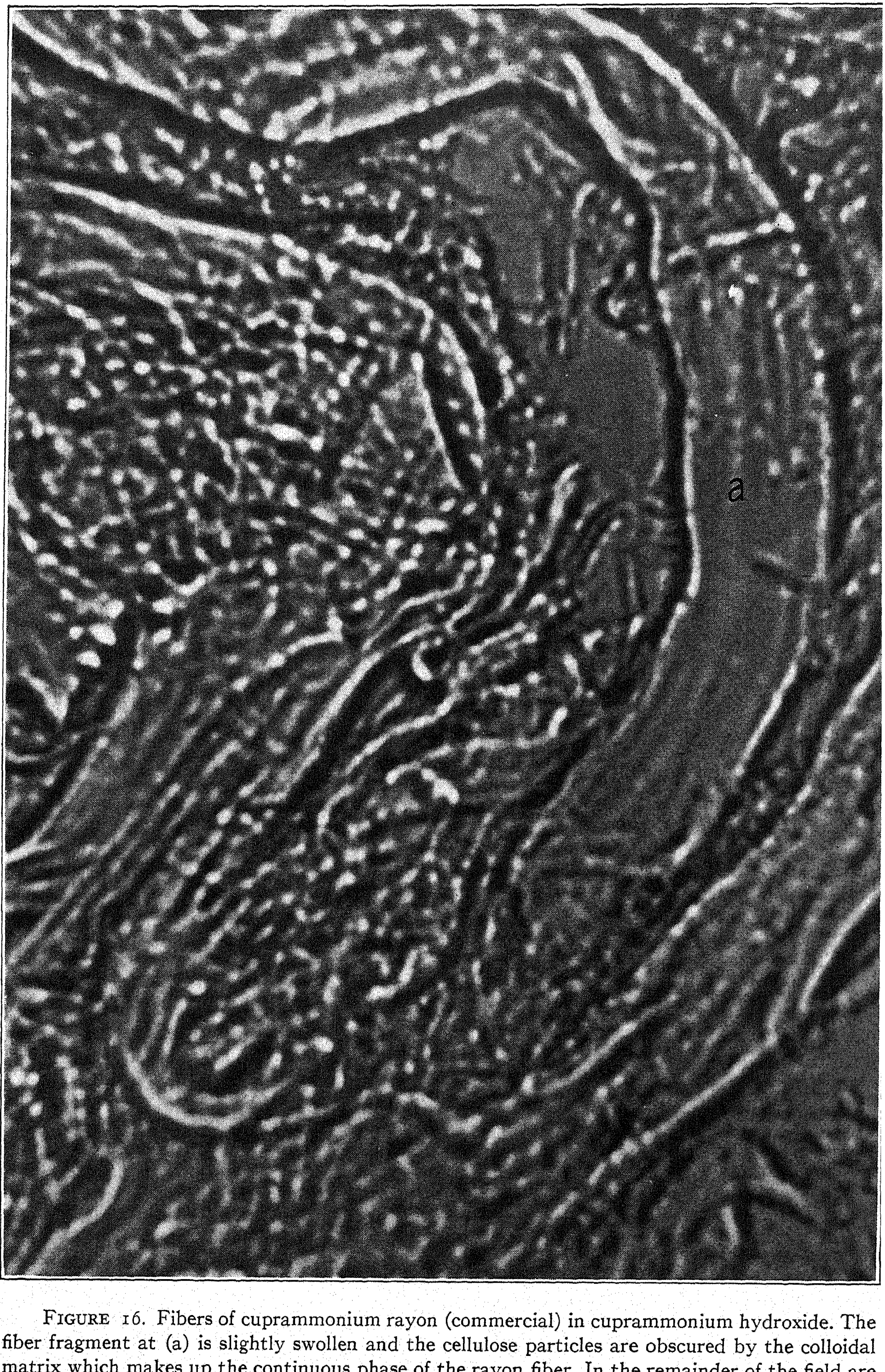

FIGURE 16. Fibers of cuprammonium rayon (commercial) in cuprammonium hydroxide. The fiber fragment at (a) is slightly swollen and the cellulose particles are obscured by the colloidal matrix which makes up the continuous phase of the rayon fiber. In the remainder of the field are fibers in advanced stages of swelling and dissolution of the matrix in which the cellulose particles are clearly visible (×1640).

microscopic ridges with the ring of cuticle in the center as a point of origin (Fig. 14 A). If the lantern-like structure is turned upon its side, the rings of cuticle at either end serve as points of origin and the pressure ridges extend out from them along the side of the lantern (Fig. 14 B).

These pressure ridges represent a microscopic phenomenon which we have found in many different gum-like substances. It can be readily illustrated with spruce gum, cherry gum, and gum arabic. A piece of gum tragacanth softened with water and subjected to pressure, which illustrates this point, is shown in Figure 15. A small mass of foreign material has served, in this instance, as a point of origin for the pressure ridges. Ambronn observed a somewhat similar microscopic phenomenon in various gums, including cherry, arabic, and tragacanth, as a result of freezing. The microscopic structures thus produced were made permanent by drying at a low temperature. He referred to them (1, p. 28) as "Pseudomorphosen von Gummi." We have found that the pressure ridges are not easily produced with swollen gelatin alone but that they are readily developed when formalin and alcohol are added to gelatin in quantities sufficient to produce the desired consistency for imbedding plant tissues. The so-called "growth rings" (4) have, therefore, no relationship to the true lamellae of the cotton fiber wall as shown by the swelling of thin cross-sections of the fibers in solutions of cuprammonium hydroxide. Their number and width have no bearing upon the size of the crystalline units of cellulose in the fiber wall and do not constitute evidence against the existence of cellulose particles of the size which we have observed and described.

The studies which have been described have been made entirely under laboratory conditions. Commercial samples of cuprammonium rayon at different stages of manufacture have not been available for examination. A microscopic examination of the commercial rayon fibers themselves at different stages of disintegration in cuprammonium solution presents a picture such as that shown in Figure 16. A fiber just beginning to break down at (a) is surrounded by pieces of fibers in more advanced stages of disintegration. In the early stage of swelling the colloidal matrix in which the cellulose particles are imbedded obscures them from microscopic visibility. In later stages the linear arrangement of particles induced presumably by ejection from the spinneret often has the appearance of a disintegrating cellulose fibril in a natural fiber membrane.

DISCUSSION

After accumulating the data which have been presented concerning the state of dispersion of the cellulose particles in the solutions of cuprammonium hydroxide, the inability of this separated cellulose component of the plant cell membrane to produce viscosities in cuprammonium hydroxide, and the viscosity-producing power of the pectic fraction of the cementing material extracted with ammonium oxalate, we were interested in reviewing again the large number of papers which have been written upon the behavior of "cellulose" in cuprammonium in search of previous suggestions that the cellulose is not in a state of solution. In this connection Peligot's early observation that the "solutions" were "not perfectly limpid" is worthy of note. The most closely related observations which we have found, however, are those of Mosenthal who published, in 1904, a microscopic and chemical study of cotton fibers, untreated and after treatment with various reagents. Assisted by Lindsay Johnson in the preparation of the microscopic mounts and in preparing photomicrographs of his material, he studied the structure of the native fiber and its structural changes after treatment with cuprammonium hydroxide, hydrofluoric acid, pepsin, tripsin, nitric acid, zinc chloride, and other reagents.

The cellulose lamellae which we have described in the untreated fiber are referred to by Mosenthal as "intercuticular substance." He states (22, p. 292): "The intercuticular substance, which, as remarked, constitutes the bulk of the fiber, consists, according to my observations, of minute spherical granules of nearly uniform size (about 1μ). I attempted to see them *in situ* by obtaining longitudinal sections of the cotton fiber. The difficulty of obtaining such sections was very great, due to the resistance of the cotton which was liable to be struck by the knife and dragged through the substance in which it was imbedded. I have, however, succeeded in obtaining longitudinally-cut fragments, some of which had one of the cuticles removed revealing the intercuticular substance (Fig. 4). The intercuticular granules lie closely packed, kept in position by the cuticles, and it is only when the tension of these is sufficiently relaxed that the granules can escape."

On page 293: "Zinc chloride both with and without the addition of hydrochloric acid destroys the structure, and neither the granules of the intercuticular substance nor the fragments of the cuticle can be found in the restored cellulose. In the cotton regained from a cuprammonium solution, the granules are preserved and the fragments of the cuticle are present."

On page 294: "If cotton fibers in course of dissolution in cuprammonium hydroxide are freed from copper and washed, it will be seen on microscopic examination that the granules gradually become freed and washed out as the cuticles yield to the swelling of the fiber. The fiber swells and bursts, thus liberating the granules, and it takes some time before the cuticles themselves break up into minute fragments and enter into solution."

On page 295: "The difficulties of the present investigation are increased by the fact that we are dealing with a substance, the structure of which is apparently partially retained in solution."

And finally on page 295: "Are we to regard these solutions as colloids, as suspensions of finely divided particles? The microscopic examination of fibers and precipitates certainly points in that direction. That no particles can be seen in the solution does not, I think, militate against that view. It is true that, according to Lottermoser, particles must be smaller than 0.5μ to be microscopically invisible in solution and our particles are much larger. But surely the detection of minute particles in a liquid does not only depend upon their size but also upon the relative refractive indices of the suspended matter and the liquid."

Our own observations and interpretations were very closely approached in this study of Mosenthal. In the course of the discussion of his paper, it became clear that he did not share with many of his contemporaries the viewpoint that the reactions of a plant cell membrane can be interpreted in terms of a single organic chemical. His closing remarks contain the statement that many members had spoken about *cellulose*, while he had spoken about *cotton*.

In dealing with the reactions of a structure such as a cotton fiber it is important that we remember that a plant cell does not come into existence, as a separate biological entity, in possession of a fully developed membrane. The materials for the formation of the membrane of the mature cell are manufactured in the living cytoplasm of the cell in the course of its vital activity. In this complex matrix small building units, to be used in the construction of the membrane, are developed by a process not yet understood. Two of their most important attributes are their small size and their physical stability. We have named these diminutive, uniform-sized structures cellulose particles because they possess all of the properties of the substance which we recognize as cellulose. It is not unlikely, in consideration of their place of origin, that even they may be found later to be chemically inhomogeneous.

The actual construction of a membrane takes place only in cells which are alive. The physical state of the cytoplasm in which the entire process takes place is that of a more or less viscous colloid. The cellulose particles during fibril formation are in the most intimate contact with this chemically complex colloidal matrix. There seems to be no reason to consider the *cementing material* anything other than a surface coating of this colloidal matrix upon each separate particle and between the successive layers of fibrils which form the wall lamellae. In interpreting the behavior of a plant cell membrane in a solution of cuprammonium hydroxide, it is necessary that the reactions of both the cementing material and the cellulose particles be given careful consideration. The remarkable physical stability of the cellulose particle is maintained even under the influence of this strong reagent. The particles are in a state of dispersion and can be observed and identified microscopically at all stages of treatment. The cotton fiber is transformed by cuprammonium hydroxide into a swollen viscous mass of cementing material in which the cellulose particles are imbedded. Neither the colloidal properties of the cementing material nor the crystalline properties of the cellulose particles are destroyed by the action of the reagent.

SUMMARY

The cellulose component of the cell membrane of the cotton fiber, which is in the form of diminutive cellulose particles, does not dissolve in the standard solution of cuprammonium hydroxide, specified by the American Chemical Society, to produce the viscosities commonly attributed to it.

The fiber is transformed by the cuprammonium hydroxide into a swollen, viscous mass of cementing material in which the cellulose particles are dispersed.

The separated cellulose particles from which the cementing material has been removed will not produce viscosities in cuprammonium hydroxide. One component of the cementing material, the pectic fraction extracted with ammonium oxalate, will produce viscosities in the same solution to the point of formation of a stiff gel.

These observations alter our previous conceptions of the behavior of both untreated and purified cell membranes in solutions of cuprammonium hydroxide. It is now clear that the viscosity-producing power of the cementing material, or of any one of its fractions, has been overlooked as has been also the presence in the cuprammonium solution of the cellulose in the form of diminutive particles of uniform size and shape, still undissolved, and merely in a state of dispersion.

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SOME OBSERVATIONS UPON THE DISPERSION, ELECTROKINETIC AND COAGULATION BEHAVIOR OF COTTON FIBERS IN CUPRAMMONIUM HYDROXIDE SOLUTION

WAYNE A. SISSON[1]

During the course of experiments upon the electrokinetic behavior of cotton fibers dispersed in cuprammonium hydroxide solution, gelatinous deposits were obtained at the anode when an electric current was passed through the mixture. When the deposit was removed from the platinum electrode or the electrophoresis cell, placed, without drying, upon a microscope slide, and examined at high magnifications, it was found to contain large numbers of uniform-sized particles. In each deposit, the particles were birefringent in polarized light (Fig. 1 D) and their cellulosic nature could be demonstrated with the sulphuric acid-iodine test, as shown in Figure 1 C. X-ray examination showed that the original native crystalline structure of the cellulose had been changed to the mercerized or hydrate form. This is in agreement with the well known fact that a mercerized structure is characteristic of cellulose recovered from cuprammonium solution by acid treatment.

The gelatinous nature of the deposit depended to a great extent upon the previous treatment of the cotton fibers. The deposits obtained from raw cotton fibers tended to form coherent membranes and the particles were less distinct, as may be seen in Figure 1 A. This is due apparently to an envelope of jelly-like material which surrounds each particle. The particle structure was best demonstrated at the edge of a clump, or by spreading out a small amount of material by pressing on the cover glass. In the deposits obtained from cotton fibers previously disintegrated with hydrochloric acid (6) the jelly-like envelope was absent, and the particle structure clearly visible, as shown in Figure 1 B.

The question arose as to whether these electrodeposited particles were the result of (a) a recrystallization of cellulose from a true solution in cuprammonium hydroxide or (b) a flocculation from a state of colloidal dispersion. In order to decide between these two possibilities it became necessary to supplement this earlier work with cataphoretic experiments and with slit ultra microscopic observations throughout the entire processes of dispersion and deposition.

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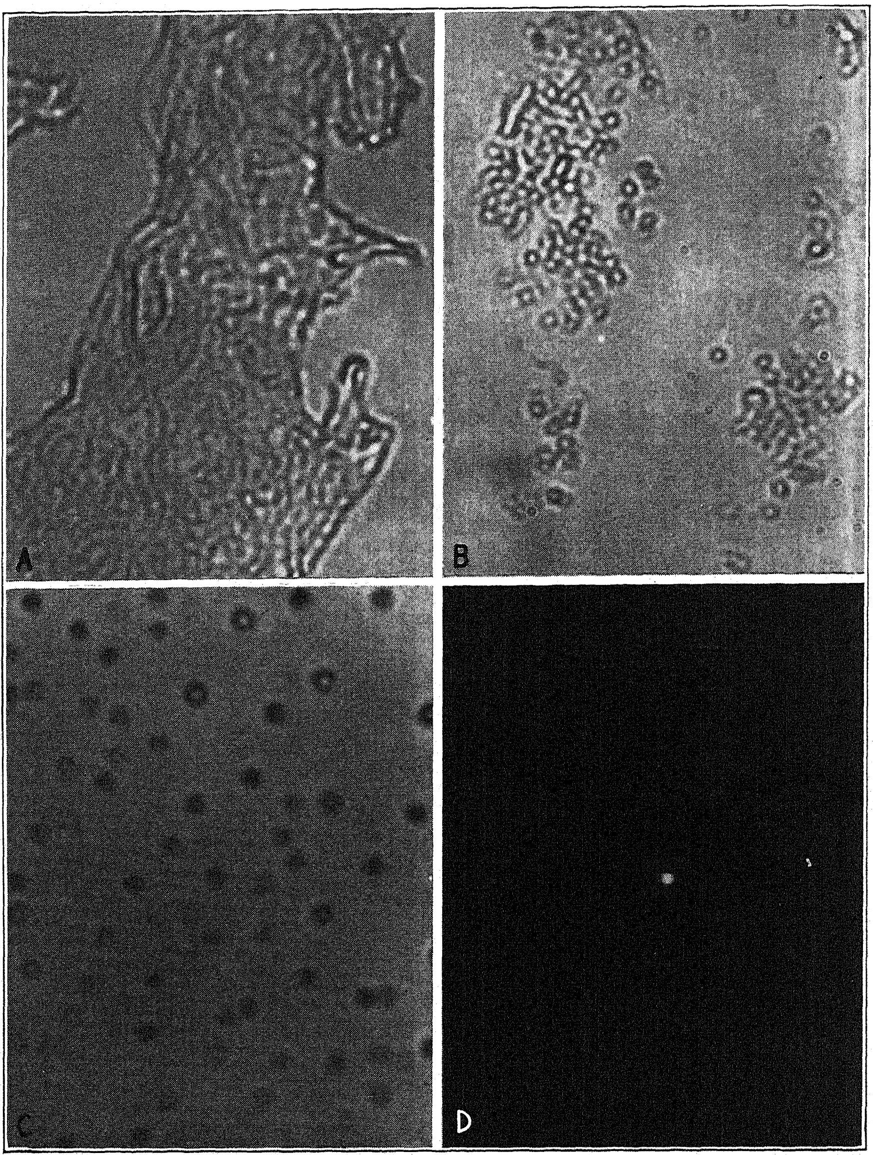


FIGURE 1. Photomicrographs of cellulose electrodeposited from cuprammonium solution. A. Material electrodeposited from untreated cotton fibers showing coherent nature of deposit and presence around each particle of jelly-like envelope which renders the particle structure less distinct (×1290); B. Material obtained from cotton fibers previously disintegrated with hydrochloric acid, showing particle structure of deposit and absence of jelly-like material around particles (×1290); C. Particles treated with iodine and sulphuric acid, showing swelling and blue color characteristic of cellulose (×1380); D. Single particle in polarized light, showing presence of double refraction (×1330).

MATERIALS AND METHODS

The samples used in the present work were untreated raw cotton fibers, kier boiled and bleached cotton fibers (1), and cotton fibers disintegrated with strong hydrochloric acid (6). Unless otherwise stated, the kiered and bleached samples were used in each experiment.

Some of the experiments, including all of the preliminary experiments on the electrodeposition of cellulose, were carried out on a stock solution of cuprammonium prepared according to the method of Clibbens and Geake (2). It contained 15 g. of copper, 240 g. of ammonia, and 1 g. of sucrose per liter. The electrolytically prepared solution used for the cataphoretic and slit ultra microscopic observations was not analyzed since the copper content changed continuously during the experiment. According to Farrow and Neale (8), who prepared similar solutions from concentrated ammonium hydroxide and copper electrodes with a current density of 0.02 amps. per $cm.^2$, the solution contains approximately 240 g. of ammonia and a maximum of 15 g. of copper per liter.

The same Leitz slit ultra microscope described by Compton (3) was used for observing the successive dispersion and coagulation of cellulose. Observations at different magnifications were made with the various combinations of 16 and 8 mm. objectives and 10 and 25× eyepieces. The microscope has a compound stage which is also adjustable in height. This permitted the microscope to be focused at various depths and positions along the cataphoresis cell which was attached to the stage of the microscope.

A camera employing 35 mm. Agfa ultra-speed panchromatic film was used for making photomicrographs with the slit ultra microscope. The X-ray diffraction patterns were made as described in earlier publications (7, 14).

The cataphoresis cell employed was similar to that described by Mattson (11) with the exception that it has two open side arms. The cell consists of a thick-walled capillary tube, 0.235 cm. inside diameter and 12 cm. long, terminating in the two side tubes. At the center are two plane polished surfaces at right angles to each other; the microscope is focused through the top surface and the illuminating beam of light enters through the side. The electrodes pass through the center of cork stoppers which are placed in the open ends of the side tubes.

RESULTS

The observations made in the present study are described in three sections: (a) electrodeposition of cellulose from cuprammonium solution, (b) the electrokinetic behavior of dispersed cellulose particles, (c) slit ultra microscopic studies regarding (a) and (b).

ELECTRODEPOSITION

Several methods of electrodepositing cellulose from cuprammonium solution are described in patents (15) which have been reviewed by Rossman (13). In the preliminary experiments of the present work, cellulose particles, held together in a gelatinous matrix, were deposited on a platinum anode immersed in a two per cent dispersion of cotton fibers in cuprammonium hydroxide solution. The electrolytic cell consisted of a 200 cc. copper cup placed on a movable support which permitted the solution to be lowered from contact with the anode after cellulose deposition had taken place. With cuprammonium solution alone, copper was

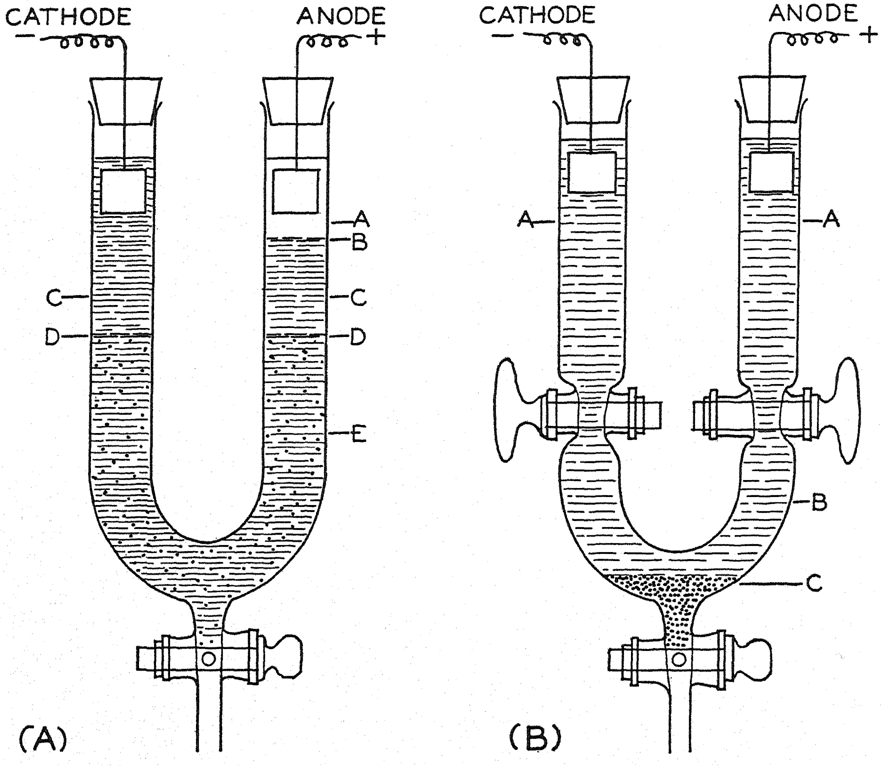


FIGURE 2. U-tubes used for studying electrical coagulation and cataphoretic behavior of cotton fibers dispersed in cuprammonium solution. (A). Coagulation apparatus, showing A ammonium hydroxide solution, B ammonium hydroxide-cuprammonium boundary, C cuprammonium solution, D boundary between cuprammonium solution and cotton dispersion, and E cotton fibers dispersed in cuprammonium; (B). Cataphoresis apparatus, showing A electrode sections of U-tube containing ammonium hydroxide, B middle section, and C disintegrated cotton fibers.

precipitated quantitatively at the cathode. At sufficiently high voltages, gas was evolved at both electrodes. With a cuprammonium dispersion of cotton fibers, a deposit suitable for microscopic and X-ray examination was obtained usually in from 5 to 10 minutes with 10 volts and 0.25 amperes. A more coherent deposit was obtained from the more viscous dispersions at higher current densities. If a very dilute dispersion was used, no deposit was formed.

The mechanism of this deposition is suggested by a second series of experiments carried out with a U-tube containing electrodes at the top and a side arm with stopcock attached at the bottom, as shown in Figure 2 (A). With cuprammonium solution alone and platinum electrodes, a clear solution A was formed at the anode with a definite boundary B between the clear ammonium hydroxide A and the blue cuprammonium solution C. This boundary B, which will be referred to as the ammonium hydroxide-cuprammonium boundary, moved toward the cathode. When the U-tube was filled completely with a cuprammonium dispersion of cotton fibers, a white deposit of the dispersed material formed around the anode as the ammonium hydroxide-cuprammonium boundary moved away. When the lower half of the U-tube was filled with a cuprammonium dispersion of cotton fibers E and the upper arms with cuprammmonium solution alone to form a boundary D between the two phases, a colorless coagulum of the dispersed material was formed at the boundary D of the two phases as the ammonium hydroxide-cuprammonium boundary B passed by. Upon replacing the platinum with a copper anode, no ammonium hydroxide-cuprammonium boundary was formed and there was no cellulose deposited in either of the above experiments.

Since a deposit may be formed at a distance from the electrode by removing the blue cuprammonium ions, this would indicate that the deposition is due not to the migration of the dispersed fiber material to the anode where its charge is neutralized by contacting the electrode, but primarily because the cuprammonium cations, which evidently are closely associated with the charge on the cellulose, are removed from the vicinity of the anode. The deposit, therefore, may be more correctly referred to as being due to electrical coagulation rather than to electrodeposition.

Materials coagulated by the above methods were subjected to both microscopic and X-ray examination. The gelatinous or non-gelatinous nature of the coagulum was again found to depend upon the treatment of the fibers previous to dispersion: the material obtained from dispersions of raw cotton consisted of cellulose particles imbedded in a coherent matrix (Fig. 1 A), while that obtained from cotton fibers previously disintegrated with hydrochloric acid was a non-coherent mass of cellulose particles in the form of brittle flakes or powder (Fig. 1 B). The reaction of the particles to sulphuric acid and iodine was positive (Fig. 1 C); they were doubly refractive in polarized light (Fig. 1 D); and their X-ray diffraction pattern was that of mercerized cellulose.

CATAPHORESIS

The presence of a negative charge on the dispersed cellulose was demonstrated by a cataphoretic experiment using the moving boundary method. The lower half of a U-tube similar to that shown in Figure 2 (A) contained a two per cent dispersion of cotton fibers, while the two upper arms contained only cuprammonium surrounding copper electrodes. Both of the dispersion boundaries [D in Fig. 2 (A)], which could be clearly observed with a strong light, moved slowly yet definitely toward the anode when a potential was applied, reversing their direction when the current was reversed. The rate of migration of the dispersion boundaries D, however, was very slow compared with that of the ammonium hydroxide-cuprammonium boundary B which forms when platinum electrodes are used as described in the previous section.

The negative charge of the cellulose phase was further demonstrated by measuring the change in concentration of the dispersed material in different sections of a U-tube. About 0.1 g. of cotton fibers previously disintegrated with hydrochloric acid (6) was placed in the middle section of a U-tube set-up similar to that shown in Figure 2 (B) and the three sections filled with concentrated ammonium hydroxide. Copper electrodes were used with a potential drop of 60 volts. The current gradually rose from 0.02 to 0.06 amperes over a period of four days. The rise in amperage was due to the dissolving of the copper anode to form a blue cuprammonium solution which migrated toward the cathode. As the cuprammonium reached the middle section B the disintegrated cotton fiber material C was gradually dispersed. After four days the solution from the two electrode sections A was removed and analyzed for cellulose by coagulating with dilute sulphuric acid. The coagulated cellulose was washed free from copper and dried at 70° C. The anode section yielded 0.0130 g. of cellulose while no cellulose could be detected in the cathode section.

When the three compartment U-tube set-up was used with a one per cent dispersion of cotton fibers in the middle section B and cuprammonium solution in the outer electrode sections A, analysis showed the anode section to contain a higher concentration of dispersed fiber material than the cathode section after a current had passed for 48 hours. A blank run without electrodes gave the same amount of material in both electrode sections. Similar results have been obtained by Neale (12).

A cataphoretic experiment was also carried out using the microscopic method on a one per cent dispersion of cotton fibers placed in a cataphoresis cell. As described in the next section, the cotton fiber disperses as small particles, the cataphoretic behavior of which may be observed in the slit ultra microscope. Under very carefully controlled experimental conditions, the particles moved toward the anode, reversing their direction upon reversing the current, indicating a negative charge.

Although the above experiments qualitatively demonstrate the behavior of dispersed fiber material, it should be pointed out that the rate of migration is very slow due to the highly ionized condition of the cuprammonium solution. Furthermore, quantitative measurements of the speed of migration were not possible by means of either the microscopic or the moving boundary method because of the counter currents set up by the rapid movement of the cuprammonium ions and the difficulty of obtaining non-polarizing electrodes.

SLIT ULTRA MICROSCOPIC OBSERVATIONS

In order to obtain more specific information regarding the nature of the dispersed cotton fiber, it was necessary to observe the dispersion, cataphoretic migration and electrical coagulation processes in the slit ultra microscope. As a matter of convenience in making the slit ultra microscopic examinations, cotton fibers were dispersed in a cuprammonium solution prepared from ammonium hydroxide in a cataphoresis cell with the aid of an electric current and copper electrodes. This special arrangement permitted the fiber sample to be dispersed and coagulated very slowly while being observed, and the coagulated sample to be removed later for microscopic and X-ray examination.

Swelling and dispersion. A small sample of fibers was placed at the center of the electrophoresis cell where all parts of the sample could be observed in the slit ultra microscope. The cell was filled with concentrated ammonium hydroxide, the side arms closed with stoppers fitted with a copper anode and a platinum cathode, and a potential of 100 volts applied. Immediately before being placed in the cell, the cotton was heated in a test tube for a minute with ammonium hydroxide solution to expel absorbed gases, the evolution of which interferes with the electrical conductivity through the capillary tube. To prohibit the evolution of gas it is also essential that the cotton sample be small, or the swollen fibers may form a compact mass which acts as a membrane. The conductivity was very low at first, but after about 24 to 36 hours the copper concentration had increased sufficiently to allow about 0.04 amps. to flow. As previously described, a definite ammonium hydroxide-cuprammonium boundary formed in the anode arm of the cell and slowly moved across the capillary tube. Fiber swelling began at low copper concentration, and as the boundary crossed the cotton fibers they began to break into smaller and smaller fragments, and finally into small particles. All stages of fiber disintegration could be observed as the fiber bundle was viewed at different positions from left to right, or if the microscope was focused in one position and the pro-

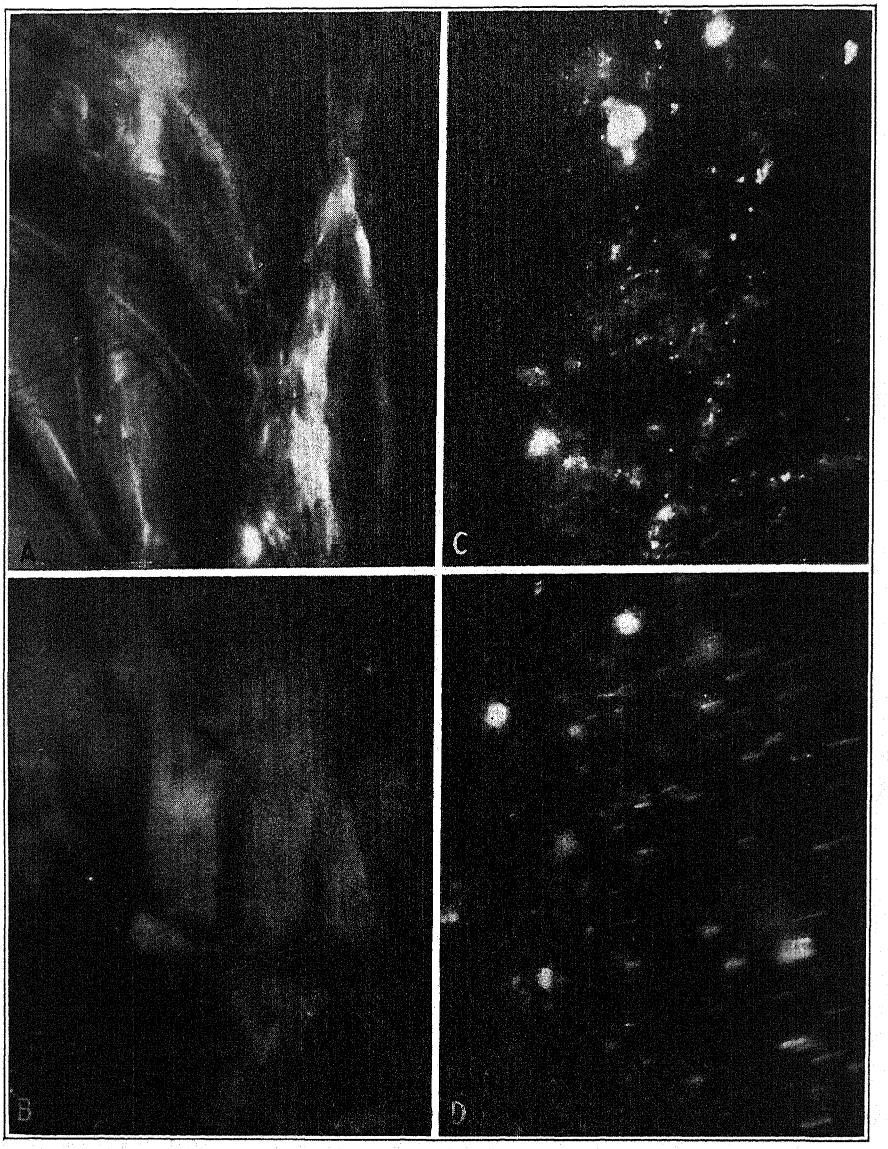


FIGURE 3. Slit ultra microscope photomicrographs showing successive stages of cotton fiber disintegration and dispersion in electrolytically prepared cuprammonium hydroxide solution. A. Cotton fibers immersed in concentrated ammonium hydroxide, showing original unswollen fibers (×127); B. Same as A after ammonium hydroxide-cuprammonium boundary has passed, showing swelling and fiber disintegration (×127); C. Later stage of fiber disintegration, showing fiber fragments of various size (×127); D. Still later stage of disintegration, showing smaller fiber fragments and cellulose particles (×127). The elongated appearance of some of the particles is due to movement during exposure.

gressive fiber disintegration observed as the boundary moved past. The unswollen fibers immersed in ammonium hydroxide are shown in Figure 3 A, while Figure 3 B shows the fiber after swelling. Under these conditions the swelling was uniform for both raw and kiered cotton and there was no formation of balloon-like structures often characteristic of cotton fibers swollen in cuprammonium solution. As the fiber fragments became smaller they gradually diffused away, assisted by the cataphoretic migration toward the anode. Figure 3 C is a view farther out from the bundle showing the presence of swollen fiber fragments. Figure 3 D shows still smaller fragments in motion. Complete fiber disintegration by the above method required from three to five days.

Dispersed state. The most common visible unit of dispersion appeared to be small uniform-sized particles of cellulose. After complete fiber disintegration the apparent number and nature of the particles did not change even after storage of the solution in an ice box for two weeks. Attempts to photograph these dispersions were unsuccessful, due to the rapid Brownian movement of the particles and the long photographic exposure necessary because of the dark blue color of the cuprammonium solution.

In order to obtain a photographic record of the particles it was necessary to prepare a dispersion of high viscosity in which the Brownian movement of the particles was greatly decreased. A two per cent dispersion of cotton fibers in a stock solution of cuprammonium was agitated until dispersion was complete. This dispersion consisted of particles which appeared to be identical with those prepared in the cataphoresis cell when examined in the slit ultra microscope. An equivalent weight of short sections of raw cotton fibers was then added to the dispersion which was quickly transferred to the cataphoresis cell, and the side arms closed without electrodes. As the raw cotton fibers swelled the viscosity of the dispersion was increased almost to that of a gel, and the Brownian movement of the particles was practically eliminated. The photographs shown in Figure 4 A and B were made by focusing at positions in the solution containing a large number of particles located between the swollen undispersed sections of raw cotton fibers. Both particles and swollen fiber sections may be seen in Figure 4 A.

Coagulation. Observations were made on the electrical coagulation of the cotton fibers dispersed in cuprammonium hydroxide solution. Coagulation was accomplished by reversing the current after portions of the fibers were disintegrated to small particles. Upon reversing the current the ammonium hydroxide-cuprammonium boundary reversed its direction leaving behind a white gelatinous coagulum containing large numbers of cellulose particles. As the copper was removed, neighboring particles cohered to form flocks or cloud-like aggregates of particles. As discussed earlier the coherence of the particles depended upon the previous treat-

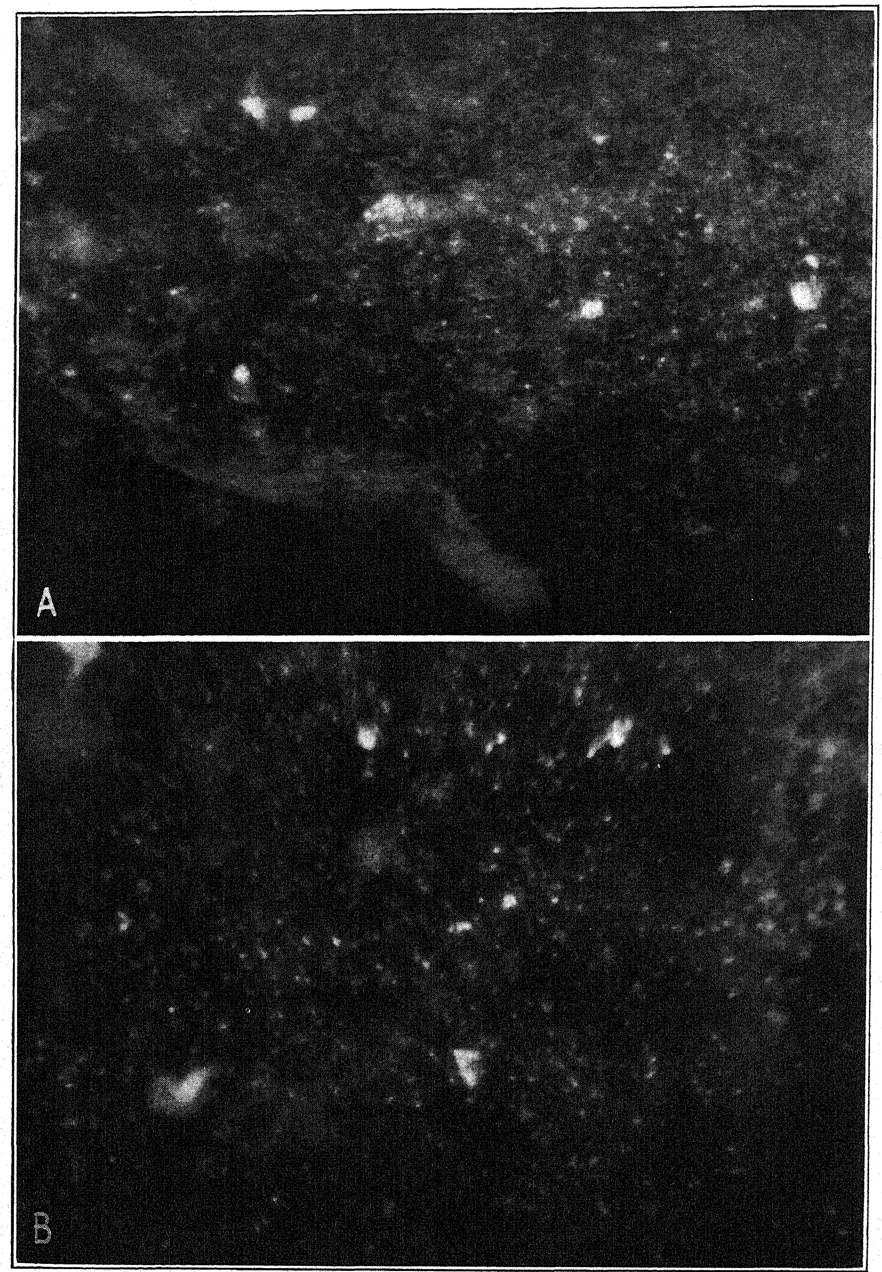


FIGURE 4. Slit ultra microscope photomicrographs showing final state of cotton fiber dispersion in cuprammonium hydroxide solution as cellulose particles. A. Cellulose particles and swollen undispersed sections of cotton fibers (×170); B. Same as A at higher magnification (×430).

ment of the cotton fibers. If the concentration of the dispersed phase was sufficiently high (2 or 3 per cent) and the solution viscous, the inside of the capillary tube was completely filled with the coagulated material.

After the particles began to adhere to each other, it was difficult to distinguish individual particles in the dark field. The glare of reflected light from each particle helped to obscure its neighbor, thus giving the appearance of a glowing nebulous mass when observed in the slit ultra microscope. Since individual particles, however, can be observed in the slit ultra microscope immediately before they coagulate, it is inferred that they are still present as individual units in the coagulum. This inference is supported by further microscopic observations of the coagulum in both ordinary and polarized light.

DISCUSSION

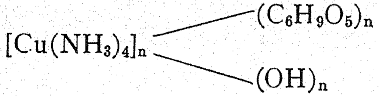
The existence of uniform-sized particles in the coagulated cellulose may be accounted for in either one of three ways: (a) the particles could be formed by a type of recrystallization from a true molecular dispersion, (b) they could be deposited from the cuprammonium solution where they existed as colloidally dispersed cellulose particles (3), or (c) they could be formed by the aggregation of submicroscopic particles or micelles. All of the evidence in the present investigation supports the second possibility. The visible disintegration of the fiber into smaller and smaller fragments and finally into uniform-sized particles, which are still present after a long period of time, would seem to indicate that most of the cellulose is colloidally dispersed as visible cellulose particles. That they are colloidally dispersed is evidenced by their Brownian movement, their electric charge and cataphoretic behavior, and the fact that they do not settle out upon standing but are flocculated upon the removal of their charge.

The present data indicate that the dispersed particles are closely related to the uniform-sized ($1.1 \times 1.5\mu$) ellipsoidal particles observed by Farr and Eckerson (5) in the cytoplasm of young cotton fibers and as final disintegration units of mature fibers treated with hydrochloric acid (6). The microscopic observations of Farr (4) regarding the swelling and dispersion of cotton fibers in cuprammonium, the quantitative particle counts of Compton (3), the present slit ultra microscopic observations regarding dispersion and coagulation, and the microscopic size of the coagulated particles all point to the same conclusion; namely, that the particle structure of the coagulated cellulose results from the original particle structure which is not destroyed during the dispersion and coagulation process.

As to why cellulose particles are colloidally dispersed in cuprammonium solution, the present evidence is only suggestive. In general, the charge on a particle may arise either (a) by adsorption of an ion or (b) by direct ionization. Since most colloids with an adsorbed ion are unstable in strongly acidic or basic solutions, it is unlikely that the charge on the cellulose particle in the strongly alkaline cuprammonium solution is due to adsorbed ions. It would seem more reasonable to assume that the charge on the cellulose particle is the result of the formation of a swelling compound with cuprammonium followed by ionization of the compound.

The formation of a swelling compound between cellulose and cuprammonium prior to, or simultaneously with, the peptization of the cellulose particle is indicated by three separate results of X-ray analysis. (a) Cellulose coagulated from cuprammonium solution possesses a mercerized crystalline structure. The action of many swelling reagents on cellulose has been followed with X-rays, but to the author's knowledge there is only one mechanism whereby cotton cellulose is changed from the native to the mercerized form; namely, by intramicellar swelling to form a swelling compound (10) followed by removal of the swelling agent. Upon swelling, the native cellulose pattern is displaced by a new pattern of the swelling compound which, in turn, changes to the mercerized pattern when the swelling agent is removed. (b) Trogus and Hess (16) have reported that cuprammonium forms a definite swelling compound with cellulose, as indicated by the appearance of a new diffraction pattern. (c) Only that portion of cellulose dispersed in cuprammonium solution gives the mercerized pattern. Cooperative work carried out with Farr (4) and Compton (3) shows that cotton fibers treated with hydrochloric acid (6), when agitated with cuprammonium, may be fractionated into two portions. One portion can be recovered by standing for a long time or centrifuging (1800 r.p.m.) for a short time, while the other remains suspended. The former gives a native X-ray pattern, while the latter, upon coagulation either electrolytically or with acids, gives a mercerized pattern. This indicates that contact with cuprammonium solution does not necessarily produce a swelling compound, and only that fraction which reacts with cuprammonium to form a swelling compound is colloidally dispersed.

The exact mechanism of the cellulose compound formation with cuprammonium and its subsequent ionization is not indicated by the present data. In order to explain the optical rotation, Hess and Messmer (9) have suggested that each glucose unit combines with cuprammonium solution to form the compound $[(C_6H_7O_5)Cu]_2 \cdot [Cu(NH_3)_4]$ which ionizes to $[(C_6H_7O_5)Cu]_2^{--} + [Cu(NH_3)_4]^{++}$. Neale (12), on the other hand, in order to explain certain electrophoretic data and the colloidal behavior of cellulose in cuprammonium solution, suggests the compound



$$[Cu(NH_3)_4]_n \begin{cases} (C_6H_9O_5)_n \\ (OH)_n \end{cases}$$

to be formed which ionizes to $(C_6H_9O_5)_n^{n-} + {}_nCu(NH_3)_4^{++} + {}_nOH^-$. Although the above reactions were originally advanced to apply to molecular and micellar dispersions, it is possible that a similar reaction might explain the peptization of the larger cellulose particle. A similar suggestion has been made by Compton (3) in order to explain the optical properties of dispersed cellulose particles.

If the above mechanism of compound formation is correct, the system may be thought of as both lyophilic and lyophobic in nature. In the fiber, the indications are that the cementing material (4) may form a true lyophilic colloid which may swell indefinitely with the dispersion medium. Cellulose particles on the other hand would appear to be lyophilic to the extent that cuprammonium solution enters into the cellulose particle to form a swelling compound, and lyophobic to the extent that during this process the particle itself is not measurably altered in size and shape.

As a tentative working hypothesis for explaining the present data, therefore, it may be assumed that the process of particle peptization is associated with the formation of a swelling compound. Upon removing the cuprammonium ions, the swelling compound is decomposed; the crystalline structure reverts to the mercerized form; and the cellulose particles are coagulated.

SUMMARY

1. Cotton fibers, when treated with electrolytically prepared cuprammonium hydroxide solution, swell and are disintegrated into small particles which disperse in the solution, as indicated by slit ultra microscopic examinations.

2. The particles exhibit Brownian movement, and possess a negative charge as indicated by their cataphoretic migration toward the anode.

3. Upon removal of the cuprammonium cations by electrolysis the particles are coagulated to form a flocculent deposit.

4. Microscopic examination shows the deposit to consist of uniform-sized cellulose particles which give a mercerized X-ray diagram.

5. The presence of particles in the deposited fiber material is attributed to a flocculation of colloidally dispersed crystalline particles rather than to the recrystallization of cellulose from a state of molecular dispersion in the cuprammonium hydroxide solution.

6. It is tentatively suggested that the peptization and change in crystalline structure of the cellulose particle is associated with the formation of a swelling compound with cuprammonium solution.

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OBSERVATIONS ON THE MEMBRANES OF EPIDERMAL CELLS OF THE AVENA COLEOPTILE

WANDA K. FARR[1] AND WAYNE A. SISSON[1]

The outer epidermal tissue of the *Avena* coleoptile has been found, by a number of investigators, to increase by means of the elongation of cells formed in the early stages of growth of the coleoptile. The following microscopic and X-ray data are concerned with the membranes of the cells of this tissue at a period during the process of cell enlargement.

Avena sativa Linn. seeds of the pure line "Siegeshafer" were obtained from Doctor E. Å. Åkerman of Svalöv, Sweden. They were germinated in moist chambers, at room temperature, under sterile conditions. The microscopic observations included in this report were made upon fresh epidermal tissue, carefully removed from the growing coleoptiles and mounted in water. No stains were employed and every effort was made to keep the cells in a normal condition during the period of study. Microscopic observations in ordinary light, of comparatively low intensity, and in polarized light, revealed the membrane characteristics described.

The delicate membranes of the very young epidermal cells of the coleoptile are composed of non-cellulosic material. Microchemical analyses show the presence of both pectic and a wax-like material, along with traces of less definitely identified components of this original limiting membrane of the protoplast. No physical differentiation has been observed in the membranes of the youngest cells. In the strips of tissue containing cells in the early stages of elongation, however, there may be seen, in various parts of the lateral walls, a tendency toward a wave-like folding (Fig. 1 A). Cells in the same region, in a slightly more advanced stage of elongation, show definitely the presence of the waved condition (Fig. 1 B). A more general aspect of the waved membranes at this stage of cell enlargement is shown in Figure 1 C. This physical state of the non-cellulosic membrane has been observed only in epidermal tissue which has been removed from the coleoptile. Its absence in membranes of young cells which are not yet beginning to elongate, its presence in the longitudinal walls and absence in the cross-walls of the elongating tissue, and the fact that it does not appear in the intact coleoptile, all point to the conclusion that the plastic walls of the elongating young cells are in a state of tension which is relieved when the epidermal tissue is stripped from the underlying layers of cells of the coleoptile.

During this early period of elongation, cellulose, in the form of visible, uniform-sized, ellipsoid particles (2, 3), accumulates in considerable quan-

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tities in the living cytoplasm. Median optical sections of the cells reveal the fact that large numbers of them are in the outer regions of the cytoplasm near to the elongating non-cellulosic membrane. A surface view of these same cells confirms this observation and shows (Fig. 2 A) the early stages of orientation of these particles in fine, transverse bands. A higher magnification of this same early stage of deposition and orientation is shown in Figure 2 B. At this stage of transverse band formation it can be observed, through careful focusing, that the cellulose particles are arranged in a single row, side by side, to form a band with their long axes parallel to the long axis of the cell. They are held together by non-cellulosic material which covers their surfaces. Figure 2 C illustrates the double refraction of the cellulose in these transverse bands and the non-double refraction of the membrane to which the bands are attached. A later stage of deposition of this first cellulosic layer in the membrane is shown in sharper focus in Figure 3. These transverse bands can be observed in the membranes of the epidermal cells of intact coleoptiles and are independent of the wrinkling action produced by stripping the epidermis from the underlying tissue.

The presence of transverse bands in the cell membranes of growing tissues has been reported by Nägeli (8), Strasburger (10, 11), Correns (1), Heyn (5), Meeuse (7), and others. Various explanations of their relationship to the structure of the membrane have been offered.

A median optical section of this same tissue at low magnification shows the continued presence of the wave-like foldings in the outer membrane (Fig. 1 C). At a focal depth between this median optical section and a surface view, at higher magnification, both the outline of the outer membrane and the transverse bands are visible (Fig. 4, A and B). This shows clearly that the plastic state of the outer non-cellulosic membrane continues after the deposition of cellulose upon its inner surface has begun.

As the elongation of the non-cellulosic membrane continues, the transverse bands of cellulose are drawn farther and farther apart (Fig. 5, A and B) without changing, however, their previous state of orientation in the membrane. After the deposition of an additional layer of cellulose upon the membrane, these widely separated bands can still be distinguished from those more recently deposited (Fig. 5 C).

The X-ray diffraction diagram of epidermal cells of the *Avena* coleoptile (2–3 cm. long) is shown in Figure 6 B. The sample was prepared by carefully peeling off strips of epidermal cells, placing them parallel to form a bundle which was allowed to dry upon a glass plate. The predominating diffraction line is that of a wax-like material and a broad, intense band which arises from unidentified amorphous material. The 002 line of cellulose (3.95 Å) is obscured by the patterns of the waxy and amorphous materials, but the 101 and $10\bar{1}$ lines (6.1 and 5.4 Å), corresponding to native cellulose, can be clearly observed in the original negative. These lines exist as two equatorial arcs which indicate that the long axes (b axis of cellulose unit cell) of the cellulose crystallites have a preferred orientation parallel to the long axis of the coleoptile. The deviation from this preferred orientation can probably be explained by disorganization which occurred during the removal of the epidermis from the coleoptile.

The X-ray diagram of oriented cellulose and a wax-like material in the *Avena* coleoptile have been reported by Heyn (6) and by Hess, Trogus, and Wergin (4). The identification of crystalline cellulose in the X-ray diffraction patterns of both their material and ours is difficult because of the presence of diffraction bands of non-cellulosic constituents. This difficulty can be overcome by extracting the non-cellulosic material with suitable reagents (9).

The X-ray diagram of a similar sample, after extraction with alcohol for 24 hours in a Soxhlet extractor and with a one per cent sodium hydroxide solution for 48 hours, is shown in Figure 6 C. The predominating pattern is now that of crystalline cellulose. Additional patterns taken at intermediate stages of the extraction show that the waxy material is removed by the alcohol and the amorphous material greatly decreased by the sodium hydroxide extraction. These extractions disturb considerably, but do not destroy completely, the original orientation of the cellulose as shown by the photomicrograph of the extracted tissue in Figure 6 A, and also by the fact that the cellulose diffraction lines are concentrated as equatorial arcs.

The fact that the non-cellulosic constituents can be removed by extraction without altering the fundamental nature of the cellulose pattern (number, intensity, and diameter of the diffraction rings) indicates that the cellulose exists as a separate crystalline phase. The presence of uniform-sized cellulose particles in transverse bands, oriented with the long axes of the particles parallel to the long axis of the epidermal cell, now makes it possible to correlate the X-ray diffraction data with a visible structure. The unchanged orientation of the cellulose during the process of cell elongation, as shown by X-ray diffraction analysis, has been difficult to explain upon the basis of a micellar structure (12). The microscopic data presented in this report show that the orientation of the visible cellulose particles is not altered during the process of cell elongation and is therefore in agreement with the X-ray data.

SUMMARY

This brief report from microscopic and X-ray diffraction studies now in progress shows that:

(a) The cellulose component of the epidermal cell membranes of the *Avena* coleoptile is in the form of microscopically-visible ellipsoid particles of native crystalline cellulose arranged in transverse bands.

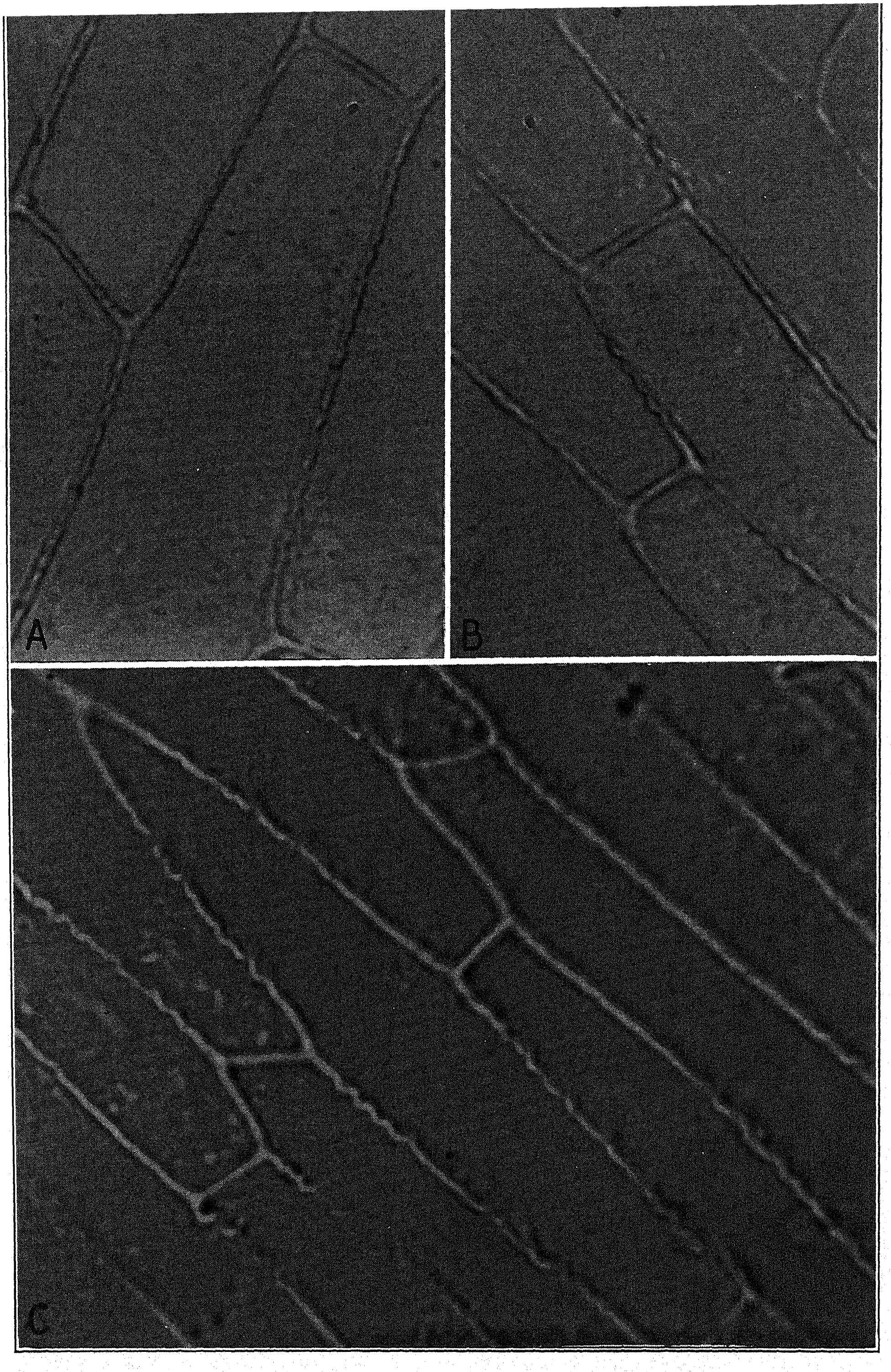

FIGURE 1. (For description see legend on page 136.)

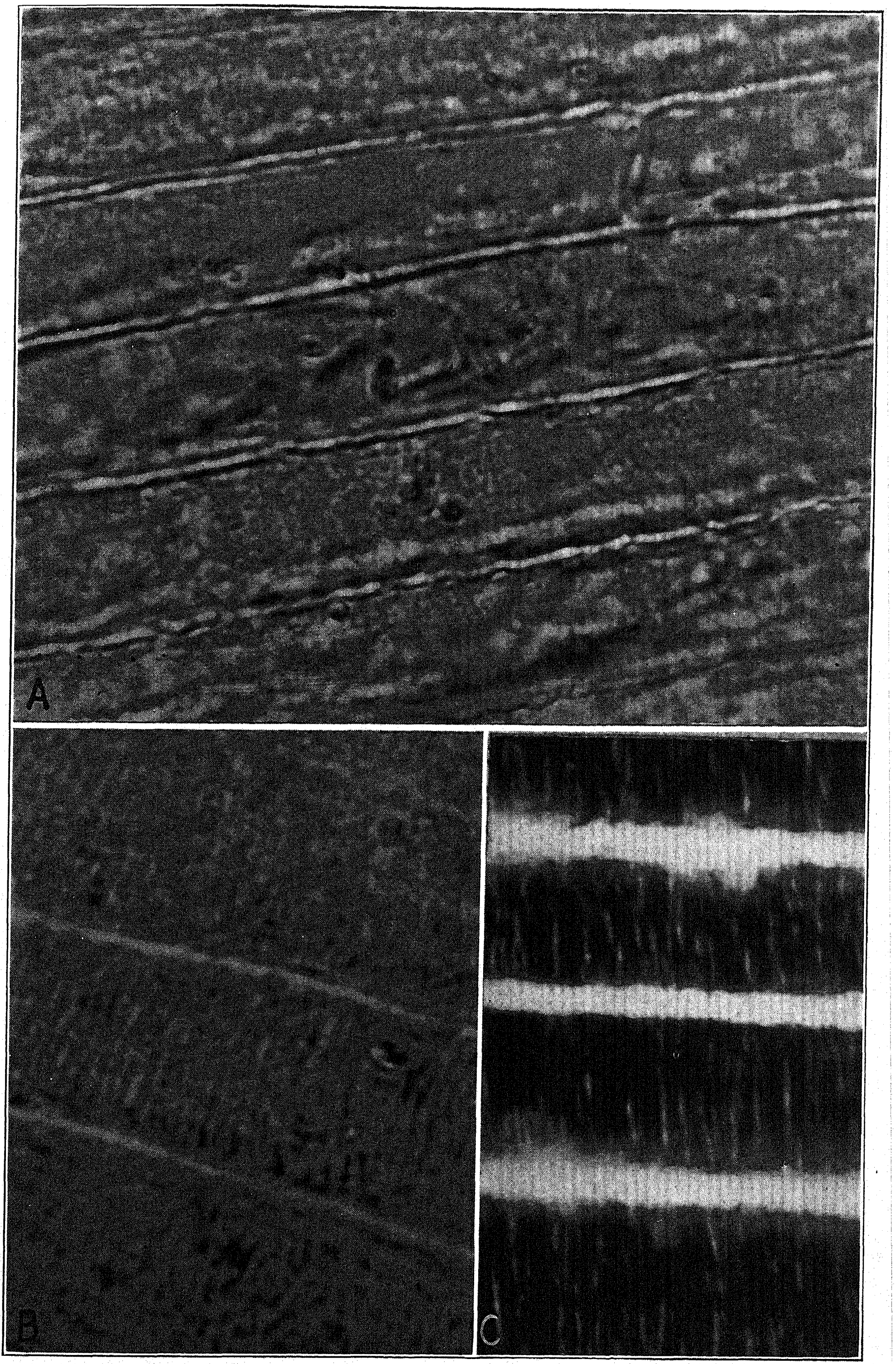

FIGURE 2. (For description see legend on page 136.)

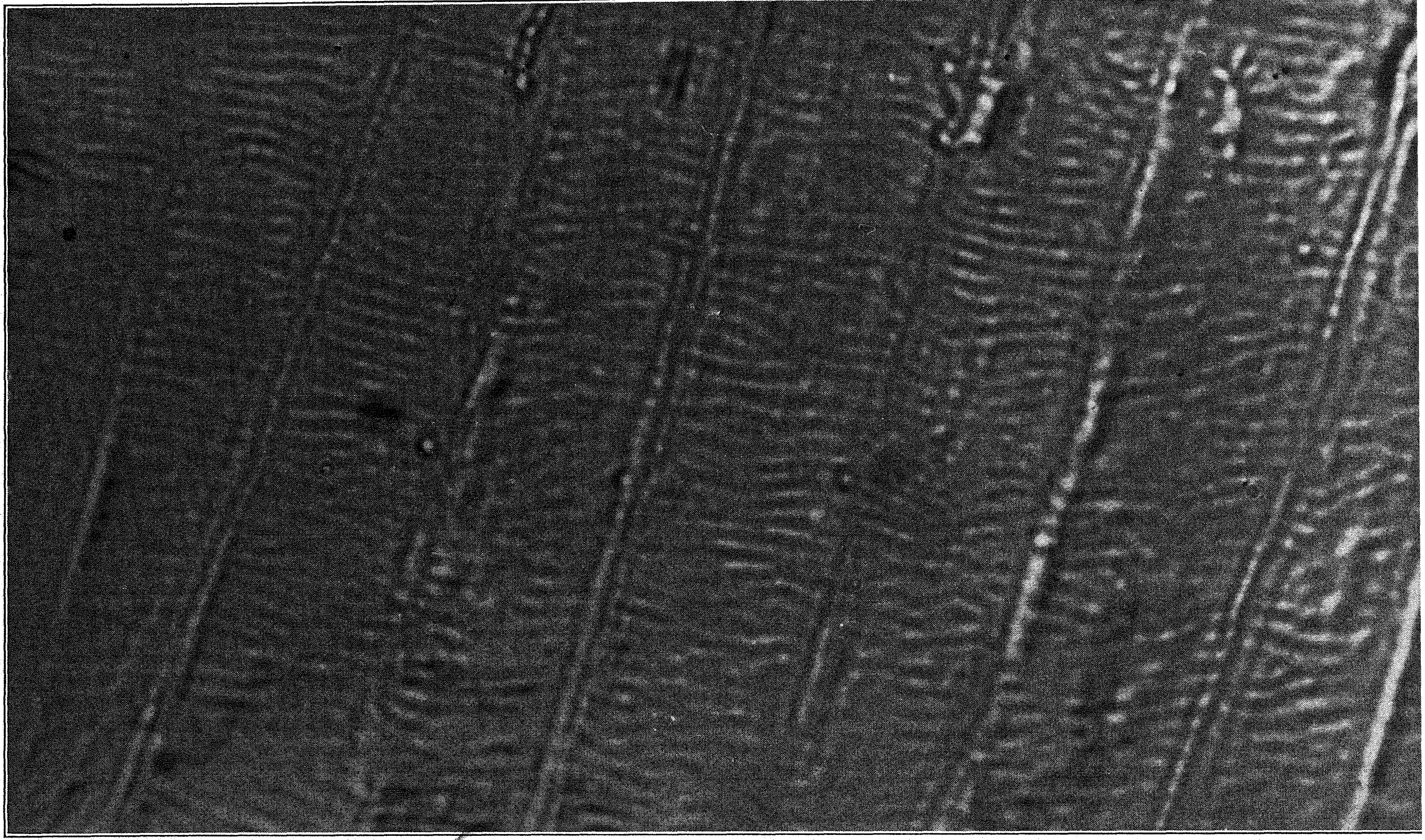

FIGURE 3. A view of the transverse bands of cellulose particles in sharper focus (×900, enlarged to 2000).

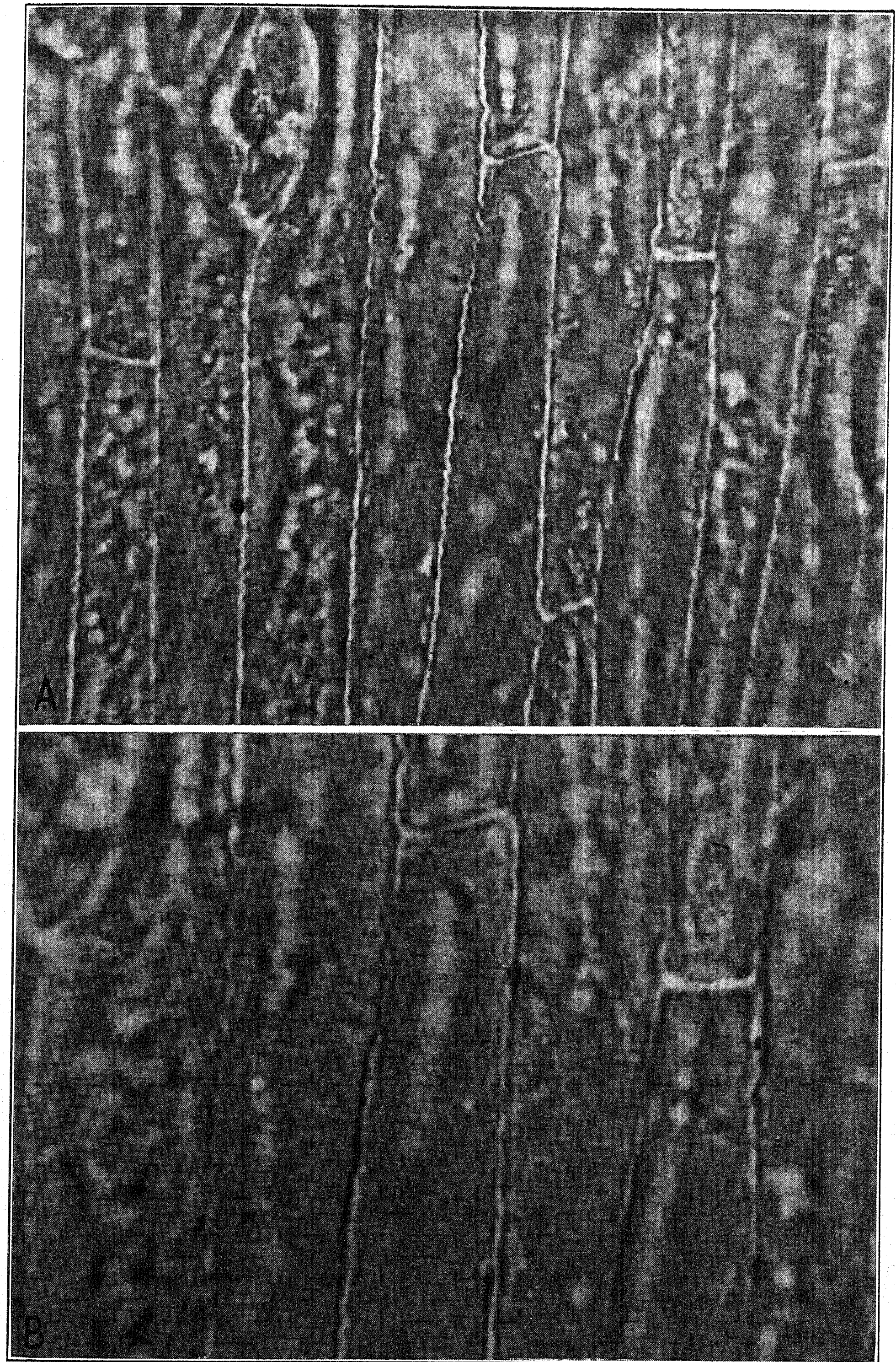

FIGURE 4. (For description see legend on page 136.)

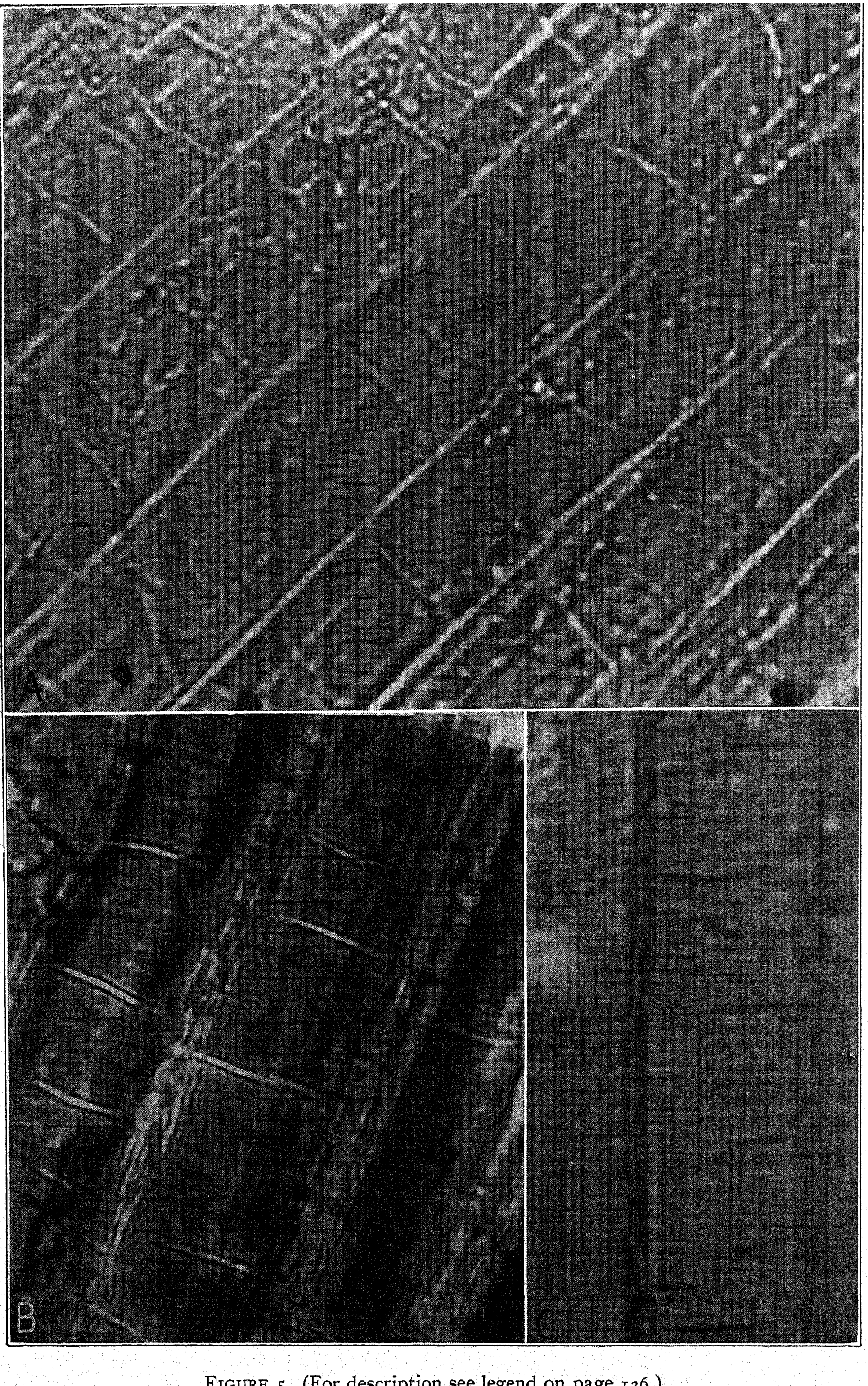

FIGURE 5. (For description see legend on page 136.)

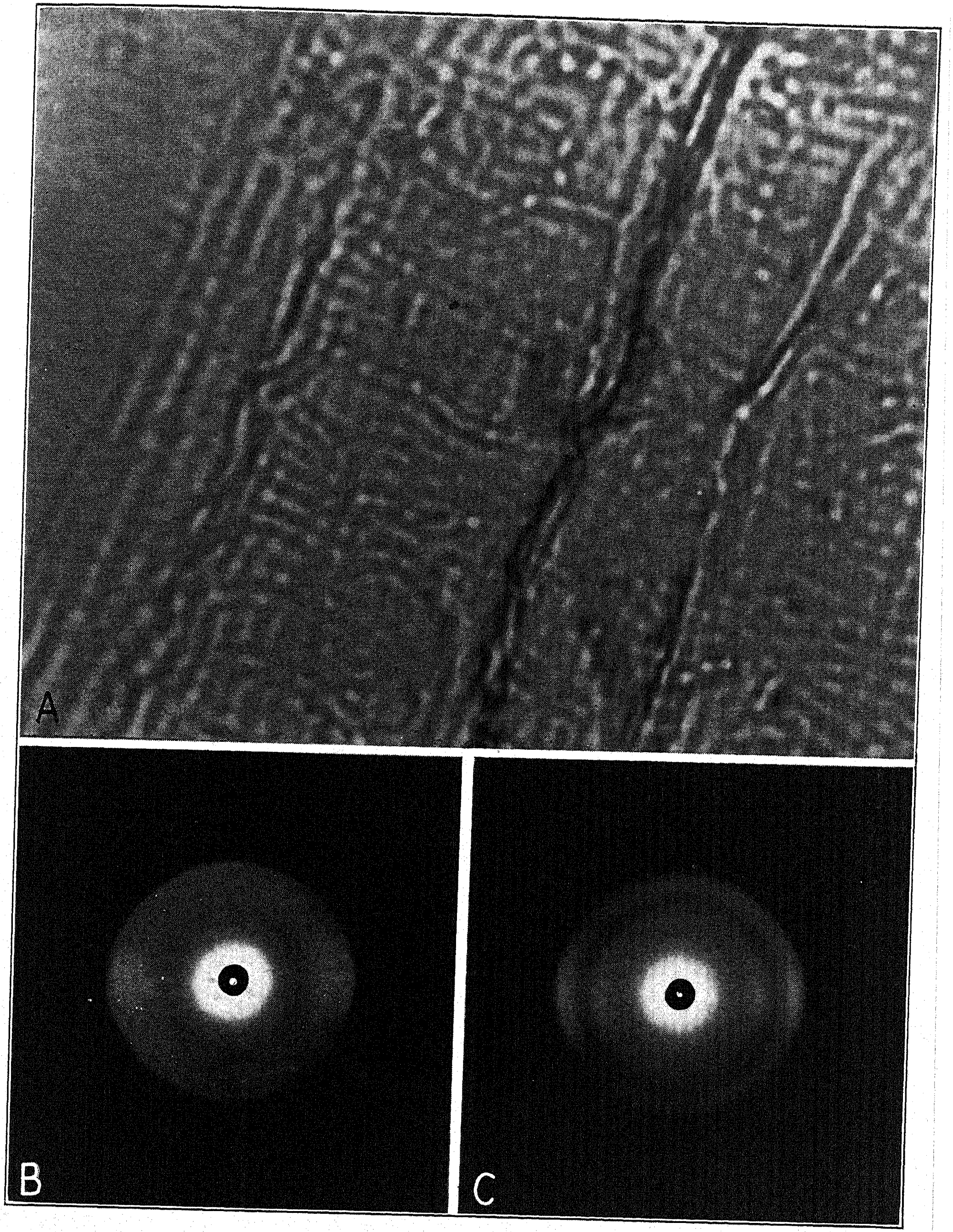

Figure 6. (A) Epidermal tissue of *Avena* coleoptile after extraction for 24 hours in alcohol and 48 hours in 1 per cent sodium hydroxide shows more clearly the cellulose particles in the transverse bands because of the removal of some of the non-cellulosic constituents (×1350, enlarged to 2100). (B) X-ray diffraction diagram of untreated epidermal cells of the *Avena* coleoptile. The long axis of the coleoptile lies in the vertical position with reference to the X-ray diagram. (C) Same as (A) after extraction for 24 hours in alcohol and 48 hours in 1 per cent sodium hydroxide.

DESCRIPTION OF FIGURES 1, 2, 4, AND 5

FIGURE 1. (A and B) Non-cellulosic membranes of young *Avena* coleoptile cells in an early stage of elongation have a waved appearance due to the release of tension when the tissue is stripped from the coleoptile (×960, enlarged to 1380). (C) This effect is general over the elongating tissue and occurs only in the longitudinal walls (×240, enlarged to 720).

FIGURE 2. (A) An early stage of deposition of cellulose particles upon the outer membrane (×690, enlarged to 1035). (B) At higher magnification the band-like cellulose depositions are seen to be made up of single rows of cellulose particles oriented with their long axes parallel to the long axis of the cell (×920, enlarged to 1380). (C) A similar stage of cellulose deposition in polarized light shows the double refraction of the cellulose bands and the non-double refraction of the membrane to which they are attached (×900, enlarged to 1380).

FIGURE 4. (A) The plastic nature of the original cell membrane is maintained after cellulose deposition has begun as shown by the wave-like contractions when tension is relieved as a result of removing the tissue from the coleoptile (×460, enlarged to 800). (B) A portion of the field in (A) at higher magnification shows the presence of both the transverse bands of cellulose and the waved non-cellulosic membrane (×690, enlarged to 1035).

FIGURE 5. (A) A later stage of cell elongation shows that the transverse cellulose bands have been widely separated by the extension of the plastic, non-cellulosic membrane (×900, enlarged to 1380). (B) A field similar to (A) in polarized light shows the double refraction of the cellulose in the separated bands and the non-double refraction of the elongating non-cellulosic membrane (×900, enlarged to 1500). (C) After the deposition of an additional layer of cellulose in the wall, these widely separated bands of the first layer are still distinguishable (×900, enlarged to 1500).

(b) During the process of cell elongation the separated bands of cellulose are seen to be distributed at increasing intervals along the membrane without alteration of their orientation with respect to the long axis of the cell.

(c) The plastic state of the non-cellulosic component of the membrane is maintained during the period of cell elongation. This is shown by the separation of the transverse bands of cellulose particles and by the wave-like foldings along the lateral walls when the tension under which they are held in the growing coleoptile is relieved.

(d) Although it has been previously supposed that the cellulose in the elongating membrane of the epidermal cell of the *Avena* coleoptile is in a state of tension, this is rendered highly improbable since the cellulose is present in the form of discontinuous bands. It is now shown that during the process of cell elongation, the tension is exerted not upon the cellulose, but upon the more plastic non-cellulosic, continuous phase of the membrane.

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SYNTHESIS OF β-(2-CHLOROETHYL)-*d*-GLUCOSIDE BY POTATO TUBERS TREATED WITH ETHYLENE CHLOROHYDRIN[1]

LAWRENCE P. MILLER

The results of tests with emulsin have shown that both *Gladiolus* corms and potato tubers (*Solanum tuberosum* L.) form a chlorine-containing β-glucoside (6) from the ethylene chlorohydrin absorbed when the tissues are treated with chlorohydrin in order to break the rest period (2, 3, 4). The acetylation of purified preparations from gladiolus corms containing the glucoside formed from the absorbed chlorohydrin has given a crystalline acetyl derivative which was found to be the tetraacetate of β-(2-chloroethyl)-*d*-glucoside (7). It seemed desirable to characterize definitely the glucoside formed by the potato as well since the potato and gladiolus are not closely related botanically and the glucosides formed by the respective tissues might not be identical. The acetylation of preparations of the glucoside from potato tubers, however, yielded the same tetraacetate as had been obtained from gladiolus corms, thus showing that both potato tubers and gladiolus corms form β-(2-chloroethyl)-*d*-glucoside from the absorbed ethylene chlorohydrin.

EXPERIMENTAL

Dormant tubers of the Bliss Triumph variety which had been treated with ethylene chlorohydrin in another experiment were used. The whole tubers were exposed in a closed container to a quantity of 40 per cent ethylene chlorohydrin vaporizing from cheesecloth equivalent to 15 cc. of a 0.1 molar solution per 100 g. of tissue for periods varying from one to seven days. At the end of the treatment period a section representing approximately one-quarter of each tuber was cut out and used for a determination of the amount of chlorohydrin which had been absorbed. The portions not used for the analyses were then stored in paper bags at 10° C. for about three weeks. After this interval no unaltered chlorohydrin remained in the tubers. The tubers were then run through a food grinder, using a fine cutter, and the juice was extracted by squeezing through cheesecloth. The tissue residue was soaked in distilled water and again squeezed through cheesecloth. The extracts obtained in this way were allowed to stand for a short period to allow the starch to settle out after which they were carefully poured off, heated to 80° C., and filtered. The filtered extracts were evaporated *in vacuo* to about one-fifth to one-seventh of their volume and were then shaken with two volumes of acetone. The

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[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 181.

mixture separated into two layers and analyses showed that most of the non-ionic chlorine (as determined by the difference in the amount of chloride obtained by an open Carius method with and without previous heating with an equal volume of N KOH) was found in the aqueous acetone layer. A number of such aqueous acetone solutions having a total volume of 4000 cc. and containing 50 millimols of non-ionic chlorine were combined and evaporated under vacuum to a volume of 160 cc. This concentrated extract was then shaken with two volumes of acetone which removed 11 millimols of non-ionic chlorine The residue was further extracted with two 200 cc. portions of hot acetone and four 200 cc. portions of hot 90 per cent acetone. For further purification the last six acetone extracts (the first extract was not used since it was considerably darker in color and presumably contained more impurities) containing a total of 18 millimols of non-ionic chlorine were combined and evaporated under vacuum to remove the acetone. Water was then added and the solution extracted a number of times with ethyl ether. The addition of a few drops of a concentrated solution of lead acetate showed the absence of substances precipitated by lead but considerable precipitation resulted from the addition of an excess of mercuric acetate. After filtration the excess mercury was removed by precipitation with H_2S, the mercuric sulphide removed by filtration, and the excess H_2S driven off by bubbling nitrogen through the solution. The solution was then evaporated under vacuum to a sticky consistency and extracted twice with 50 cc. portions of hot acetone and twice with 50 cc. portions of hot 98 per cent acetone. The combined acetone extracts, containing 11 millimols of non-ionic chlorine, were then evaporated to a very low volume under vacuum and evaporated again after the addition of some absolute ethyl alcohol. This was repeated a number of times to drive off the last traces of water. About 25 cc. of dry pyridine were then added and the mixture evaporated further to remove the ethyl alcohol. Additional pyridine was added to make a total volume of about 100 cc. and after the addition of 60 cc. of acetic anhydride the acetylation was allowed to proceed at room temperature overnight.

The acetylated mixture was then poured into about five times its volume of ice water and the product of the reaction obtained as described previously for gladiolus (7, p. 426). The crystalline material remaining after the evaporation of the chloroform was dissolved in hot absolute alcohol and clarified with Norite. The first crop of crystals obtained weighed 2.65 g. and melted at 115° to 115.5°. A second and third crop had a total weight of 0.96 g. and melted at 107° to 111°. After three more recrystallizations from hot absolute alcohol, using only the first crop of crystals in each instance, 1.27 grams melting at 117.5° to 118° (corrected for stem exposure) were obtained. Optical rotation was found to be $[\alpha]_D^{26} - 21.9°$ in acetone (concn., 3.96 g. in 100 cc.) and $[\alpha]_D^{26} - 13.7°$ in chloroform (concn., 4.895

g. in 100 cc.) These constants compare with a melting point of 118.5° to 119.0° and a specific rotation of −21.5° in acetone and −13.4° in chloroform found for synthetic *β*-(2-chloroethyl)-*d*-glucoside tetraacetate (7). Jackson (5) reports a melting point of 118.5° to 119.5° (uncorr.) and a specific rotation of −13.7° in chloroform at 20° C. and Coles, Dodds, and Bergeim (1) a melting point of 114° (corr.) and a specific rotation of −21.25° in acetone at 28° for their preparations of this compound.

Analyses:[2] Calculated for *β*-(2-chloroethyl)-*d*-glucoside tetraacetate, $C_{16}H_{23}O_{10}Cl$: C, 46.78; H, 5.64; Cl, 8.64. Found: C, 46.88, 46.68; H, 5.56, 5.81; Cl, 8.52, 8.66.

SUMMARY

The acetylation of preparations of the chlorine-containing *β*-glucoside formed by potato tubers from absorbed ethylene chlorohydrin yielded *β*-(2-chloroethyl)-*d*-glucoside tetraacetate. The glucoside formed by the tubers is thus *β*-(2-chloroethyl)-*d*-glucoside and is identical with the glucoside formed by gladiolus corms treated with ethylene chlorohydrin.

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[2] The author is indebted to Miss H. Jeanne Thompson for performing the microanalyses

TOXICITY OF VARIOUS ESTERS PREPARED FROM CHRYSANTHEMUM MONOCARBOXYLIC ACID, THE ACIDIC PORTION OF PYRETHRIN I[1]

Edward K. Harvill

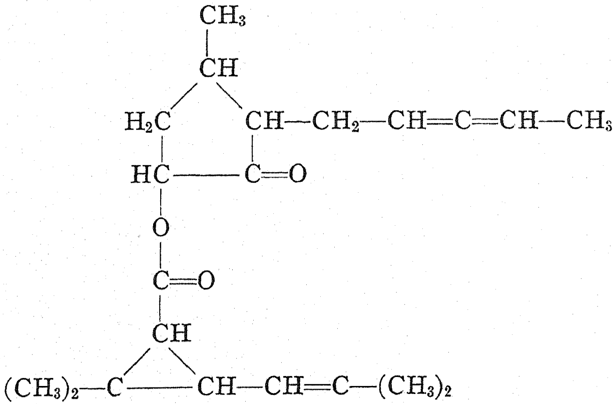
INTRODUCTION

At the present time strenuous efforts are being made to develop cheap and effective insecticides. Pyrethrum flowers, whose active constituents are toxic to insects and comparatively harmless to warm-blooded animals, constitute one of our best contact insecticides (1).

There are several disadvantages in the use of the pyrethrins. Production is controlled to a very large extent by hand methods in one or two countries and due to present conditions the price of this material has increased and the quality decreased. Furthermore the toxic principles are fairly unstable and tend to deteriorate on standing (2). In the present paper a study has been made of the toxicity of esters of chrysanthemum monocarboxylic acid (2,2,dimethyl-3-isobutylene-trimethylene-1-carboxylic acid), the acidic portion of the pyrethrin I ester. The stability of these esters has also been noted.

HISTORICAL BACKGROUND

In 1924 Staudinger and Ruzicka showed that the toxic agents in the pyrethrum flowers were the two esters called pyrethrin I and pyrethrin II (4).



Pyrethrin I

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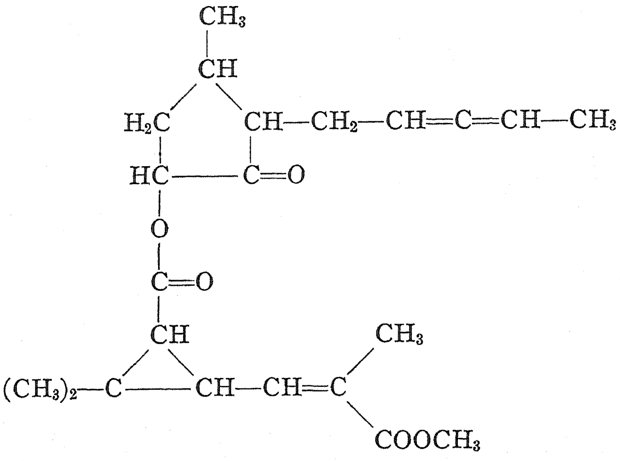
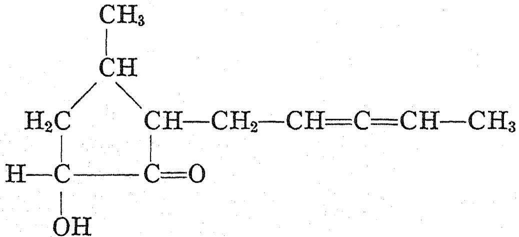
[1] A portion of this work was submitted in partial fulfillment of the requirements for the degree of Master of Science at New York University.

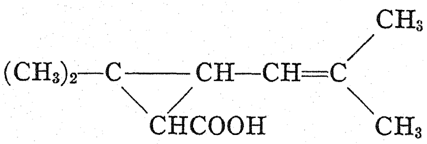


Pyrethrin II

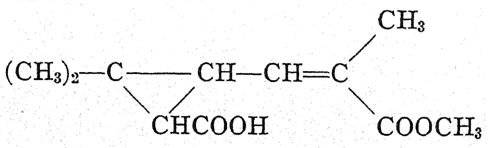
Pyrethrolone (I), the alcoholic portion, was shown to be common to both pyrethrins (6).



(I)



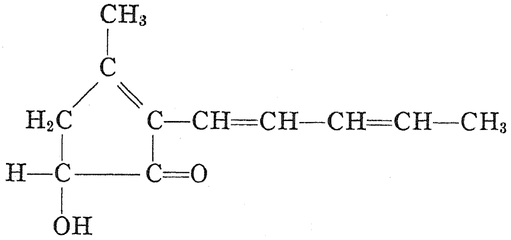
(II)



(III)

The acid fraction in pyrethrin I was found to be chrysanthemum monocarboxylic acid (II). Chrysanthemum dicarboxylic acid monomethyl ester (III) was combined with (I) in pyrethrin II (5).

Later, LaForge and Haller (3) on the basis of further investigation showed the identity of tetrahydropyrethrone and dihydrojasmone and revised the structure of (I) to (IV) as follows:



(IV)

The position of the double bonds in the side chain has not been definitely proven as yet.

Besides their structural work, Staudinger and Ruzicka attempted to replace the acidic and alcoholic portions of the pyrethrins with various alcohols, phenols, and acids and synthesized substituted cyclopentenolone, cyclopentanolone, and trimethylene compounds that approximated closely the structures of (I) and (II). These compounds were esterified with pyrethrolone or chrysanthemum monocarboxylic acid (7). The resulting esters were tested upon insects for the typical pyrethrin action (2). Staudinger and Ruzicka showed that of the hundred or more esters formed very few were found to give the characteristic intoxicating pyrethrin action or to have any toxicity. However, a study of their work reveals the fact that in no instance was a specific compound isolated and identified, the assumption being that reaction had taken place with the formation of the desired ester.

DISCUSSION AND METHODS

Although the pyrethrins contain the essential groupings pyrethrolone, chrysanthemum monocarboxylic acid, and chrysanthemum dicarboxylic acid, it was decided to work only with the mono acid in order to facilitate the experimental work. Pyrethrolone is a high boiling unsaturated ketone alcohol, unstable and difficult to obtain. Chrysanthemum dicarboxylic acid is easily secured but previous work has shown that only the mono methyl ester forms a toxic compound when esterified with pyrethrolone. The monomethyl ester is difficult to extract in suitable quantities. However, the mono acid forms a toxic pyrethrin with pyrethrolone and since it can be secured fairly easily the entire experimental work has been confined to the formation of esters of this acid with various alcohols.

Furthermore, the work of Staudinger and Ruzicka points to the mono acid as the toxiphoric grouping in the pyrethrins. Esters of pyrethrolone with various acids failed to produce a toxic compound unless the acidic portion contained a trimethylene structure and an unsaturated side chain. Several toxic esters were secured, however, when alcohols or phenols that were entirely different structurally from pyrethrolone were esterfied with the mono acid.

Preliminary work with furfuryl alcohol, α-naphthol and vanillin failed to produce an ester that was significantly toxic. Applied to the cockroach (*Periplanela americana* L.) none of these compounds produced the characteristic pyrethrin action although the vanillin ester has been reported to produce these symptoms two hours after application (7). The furane group in furfuryl alcohol and the naphthalene group in α-naphthol caused no great increase in toxicity in these esters. The addition of an amino grouping in diethanolamine to the mono acid yielded a compound that gave a fairly good kill towards *Aphis rumicis* L. but which did not induce the pyrethrin symptoms in roaches.

A more detailed study was then made of the effect of alkane groups of various lengths and structures. For this purpose a series of aliphatic alcohols of different carbon content were used and in several cases secondary and tertiary alcohols were esterified to determine the effect, if any, of the different structures. A total of 18 alcohols starting with ethyl alcohol and concluding with ceryl alcohol were esterified with chrysanthemum monocarboxylic acid, and the resulting compounds were tested for insecticidal value.

The compounds were identified in most cases by microanalytical determinations of carbon and hydrogen. In the lower series of alcohols the ester was formed by heating silver chrysanthemumate and an alkyl iodide. The method was direct and gave almost theoretical yields. Ethyl chrysanthemumate was hydrolyzed and sodium chrysanthemumate was separated and identified. Carbon and hydrogen analyses identified several of the lower esters showing that the reaction proceeded as was expected. Where other procedures were used carbon and hydrogen were determined to check with the theoretical values. Most of the esters had a high boiling point and decomposed when distilled under a high vacuum. Wherever possible boiling points and melting points were determined.

INSECT TOXICITY TESTS

In the toxicity tests the method of Hartzell and Wilcoxon was used (2). A nasturtium plant (*Tropaeolum majus* L.) carrying 100 to 200 aphids was sprayed with the solution. A solution containing 0.03 per cent of the ester and 0.5 per cent of a spreading agent (Penetrol) in water was used in every test. The aphids were sprayed with an atomizer and compressed air at a pressure of 40 cm. of mercury. The plants were left in the greenhouse for 24 hours after which they were counted, following exactly the technique of Hartzell and Wilcoxon. The results of these tests are given in Table I.

TABLE I

TOXICITY OF VARIOUS ESTERS OF CHRYSANTHEMUM MONOCARBOXYLIC ACID

Ester	% kill of *Aphis rumicis*
Ethyl	37.0
n-Propyl	26.6
Isopropyl	24.3
n-Butyl	31.4
Secondary butyl	18.0
Tertiary butyl	22.0
Isobutyl	25.0
n-Amyl	21.5
Isoamyl	14.4
Tertiary amyl	28.3
Hexyl	18.6
Octyl	57.6
n-Decyl	22.5
Undecyl-2	48.8
Lauryl	60.4
Myristyl	62.0
Cetyl	65.3
Ceryl	26.9
Furfuryl	38.7
Diethanol amine	63.7
Vanillin	59.3
α-Naphthol	14.5
Control	17.9
Pyrethrins	70.0

With the exception of the octyl ester which behaves abnormally, the lower members of the series showed a toxicity only slightly above that of the control. In these cases the percentage of kill is the average of two runs. With an increase in the carbon content the toxicity of the ester increased for the series lauryl, myristyl, and cetyl where the kills were 60.4, 62.0, and 65.3 per cent respectively and the results are the average of three or more runs. The increase did not continue with longer chains in the case of ceryl alcohol. From the dosage-mortality curve of Hartzell and Wilcoxon (2) the pyrethrins at a concentration of 0.03 per cent were found to have a 70 per cent kill when aphids were used. An examination of Table I shows that the toxicity of the esters containing alcohol groups of from 12 to 16 carbon atoms compares favorably with that of the pyrethrins. Direct application of these compounds to various portions of the bodies of cockroaches failed to produce characteristic pyrethrin symptoms. Evidently the action of these esters on insects is entirely different from that of the naturally occurring compounds or else the symptoms are too weak to be recognized.

As soon as these esters were prepared in the laboratory they were placed in glass vials and kept in a cabinet without further attention. Over a period of six to eight months the compounds retained their original color, odor, and refractive index except for the furfuryl and vanillin compounds. The furfuryl ester turned from a golden-yellow liquid to a brownish-black oil within two weeks. The vanillin compound when first made was a thick liquid which changed to a non-flowing resin in several days. Particular interest was paid to cetyl chrysanthemumate and diethyl chrysanthemumate amine since these esters showed the greatest toxicity. No physical decomposition was noted during storage in the laboratory. The change in toxicity was investigated by spraying aphids with solutions of these esters when they were freshly prepared and again after they had stood for at least six months. The results are given in Table II.

TABLE II

TOXICITY OF ESTERS WHEN FRESHLY PREPARED AND AFTER SIX OR MORE MONTHS OF STORAGE

	% kill of *Aphis rumicis*	
	Oct. 29, 1937	Sept. 14, 1938
Diethanol amine ester of chrysanthemum monocarboxylic acid	60.1	63.6
	March 24, 1938	Sept. 20, 1938
Cetyl chrysanthemumate	63.7	64.5

Evidently substitution of pyrethrolone by an alcohol such as diethanol amine or cetyl alcohol produces a stable ester that undergoes little physical change or loss of toxicity over a period of six or more months.

PREPARATION OF ESTERS

Experimental work. Chrysanthemum monocarboxylic acid was used as the starting material in the formation of the following esters and was secured from a concentrated petroleum ether extract of pyrethrum flowers. Since the extract is quite expensive the most efficient method of making the ester was used wherever it was possible. This consisted in heating the silver salt of the acid with an alkyl iodide. Three procedures were used in all which are designated by the following letters.

Procedure A—1.6 g. of silver chrysanthemumate and an equivalent amount of the alkyl iodide were heated on the steam bath. The mixture was then extracted and washed thoroughly with petroleum ether. The ether solution was dried over anhydrous sodium sulphate and the ether was then evaporated off. The resulting ester was fractionally distilled. Where the esters were too high boiling to distill, an excess of the silver salt was used and the ester was secured in a pure state without distillation. In the case of the tertiary alcohols it was necessary to wash the petroleum ether solution of the ester with dilute sodium thiosulphate to remove the iodine formed by decomposition of the iodide.

Procedure B—1.5 to 2.0 g. of the chrysanthemum acid chloride, 3.0 g. quinoline and an equivalent amount of the alcohol were refluxed in petroleum ether for one hour and then allowed to stand for two days at room temperature. The solution was filtered from the quinoline hydrochloride and washed thoroughly with dilute acid and alkali and then with water. The petroleum ether solution containing the ester was dried over anhydrous sodium sulphate. The ether was evaporated off and the compound left behind was dried in a vacuum oven at 70° for at least 24 hours or until the microanalyses for carbon and hydrogen would check with the theoretical values. The esters were difficult to secure dry.

Procedure C—1.5 to 2.0 g. of chrysanthemum acid chloride were added drop by drop with constant stirring to a 10 per cent sodium hydroxide solution containing an equivalent amount of the alcohol or phenol. The solution was boiled for 10 minutes under a reflux and allowed to stand at room temperature overnight. The solution was then extracted with petroleum ether. The ether solution was washed with dilute acid, alkali, and water and dried over anhydrous sodium sulphate. The ester was secured by evaporating off the petroleum ether.

Extraction of chrysanthemum monocarboxylic acid. Fifty grams of a concentrated petroleum ether extract containing 20 per cent pyrethrins were refluxed for one hour in 200 cc. of 95 per cent alcohol containing 7 grams of sodium hydroxide. One liter of water was then added and the solution was heated on a steam plate until most of the alcohol was driven off. "Celite" and 100 cc. of a 10 per cent $BaCl_2$ solution were added to the cooled mixture. This material was then filtered and acidified by adding 20 cc. of concentrated sulphuric acid. Low boiling petroleum ether was used to extract the acid. The ether solution was dried over anhydrous sodium sulphate overnight. The acid was secured by evaporating off the petroleum ether and distilling the acid under a high vacuum.

Yields 1.8 to 2.0 g./50 g. of extract
B. p. 156 to 157°/32 mm.
114 to 115°/2.0 mm.

The acid chloride was made by refluxing the acid with an excess of thionyl chloride.

B. p. 77°/1.5 mm.
Amide—m. p. 129° to 130° C.

Calculated for $C_{10}H_{15}ONH_2$	Carbon = 71.85%	Hydrogen = 10.17%
Found	Carbon = 71.83%	Hydrogen = 10.36%

Anilide—m. p. 99°

Ethyl chrysanthemumate—Prepared by procedure A.

B. p. 214° C.
Ref. index 1.4610/29° C.

A half a gram of the ester was hydrolyzed with 15 cc. of 10 per cent sodium hydroxide for two and one-half hours. The cooled solution precipitated silky crystals that were filtered, thoroughly washed with petroleum ether, and dried in a vacuum desiccator. A residue determination was made.

Calculated for $C_{10}H_{15}O_2Na$	Na = 12.10%
Found	Na = 12.39%

n-Propyl chrysanthemumate—Procedure A.

B. p. 234°
Ref. index 1.4601/29° C.

Isopropyl chrysanthemumate—Procedure A.

B. p. 81–82°/1.5 mm.
Ref. index 1.4549/29°.

Calculated for $C_{13}H_{22}O_2$	Carbon = 74.28%	Hydrogen = 10.47%
Found	Carbon = 74.35%	Hydrogen = 10.60%

n-Butyl chrysanthemumate—Procedure A.

B. p. 97°/1.5 mm.
Ref. index 1.4577/29°

Calculated for $C_{14}H_{24}O_2$	Carbon = 75.00%	Hydrogen = 10.70%
Found	Carbon = 74.75%	Hydrogen = 10.35%

Secondary butyl chrysanthemumate—Procedure A.

B. p. 236.5°
Ref. index 1.4607/29°

Tertiary butyl chrysanthemumate—Procedure A.

B. p. 216 to 218° (starts to decompose at 210°)
Ref. index 1.4762/29°

Isobutyl chrysanthemumate—Procedure A.

B. p. 98°/1.5 mm.
Ref. index 1.4584/29°

Calculated for $C_{14}H_{24}O_2$	Carbon = 75.00%	Hydrogen = 10.70%
Found	Carbon = 75.03%	Hydrogen = 11.08%

n-Amyl chrysanthemumate—Procedure A.

B. p. 259°
Ref. index 1.4625/29°

Isoamyl chrysanthemumate—Procedure A.
B. p. 221°
Ref. index 1.4592/29°
Calculated for $C_{15}H_{26}O_2$ Carbon = 75.62% Hydrogen = 10.92%
Found Carbon = 74.81% Hydrogen = 11.11%

Tertiary amyl chrysanthemumate—Procedure A.
B. p. 130°/5 mm.
Ref. index 1.4652/29°

Hexyl chrysanthemumate—Procedure A.
Ref. index 1.4582/29°
Calculated for $C_{16}H_{28}O_2$ Carbon = 76.19% Hydrogen = 11.11%
Found Carbon = 75.81% Hydrogen = 11.46%

Octyl chrysanthemumate—Procedure B.
Ref. index 1.4619/29°
Calculated for $C_{18}H_{32}O_2$ Carbon = 77.14% Hydrogen = 11.43%
Found Carbon = 77.69% Hydrogen = 11.39%

n-Decyl chrysanthemumate—Procedure B.
Ref. index 1.4614/29°
Calculated for $C_{20}H_{36}O_2$ Carbon = 77.92% Hydrogen = 11.69%
Found Carbon = 77.19% Hydrogen = 11.65%

Undecyl-2 chrysanthemumate—Procedure B.
B. p. 152 to 153°/0.5 mm.
Ref. index 1.4599/29°
Calculated for $C_{21}H_{38}O_2$ Carbon = 78.26% Hydrogen = 11.80%
Found Carbon = 77.87% Hydrogen = 11.40%

Lauryl chrysanthemumate—Procedure B.
Ref. index 1.4616/29°
Calculated for $C_{22}H_{40}O_2$ Carbon = 78.57% Hydrogen = 11.90%
Found Carbon = 78.01% Hydrogen = 12.06%

Myristyl chrysanthemumate—Procedure B.
Ref. index 1.4626/29°
Calculated for $C_{24}H_{44}O_2$ Carbon = 79.12% Hydrogen = 12.09%
Found Carbon = 78.71% Hydrogen = 12.30%

Cetyl chrysanthemumate—Procedures A and B.
Ref. index 1.4642 Procedure A.
Ref. index 1.4638 Procedure B.
Calculated for $C_{26}H_{48}O_2$ Carbon = 79.59% Hydrogen = 12.24%
Found Carbon = 78.92% Hydrogen = 12.60%

Ceryl chrysanthemumate—Procedure B.
M. p. 46 to 47° (waxy material)
Calculated for $C_{36}H_{68}O_2$ Carbon = 81.20% Hydrogen = 12.78%
Found Carbon = 81.05% Hydrogen = 12.46%

Furfuryl chrysanthemumate—Procedure C.
B. p. 216°
Ref. index 1.4852/22°
Calculated for $C_{15}H_{20}O_3$ Carbon = 72.58% Hydrogen = 8.06%
Found Carbon = 72.81% Hydrogen = 7.78%

Diethanol amine ester of chrysanthemum monocarboxylic acid—Procedure C.
Resin
Ref. index 1.4970/29°
Calculated for $C_{24}H_{39}O_4N$ Carbon = 71.11% Hydrogen = 9.63%
Found Carbon = 71.10% Hydrogen = 9.64%
" 70.85% " 9.66%

Vanillin chrysanthemumate—Procedure C.
Ref. index 1.5267/29°
Calculated for $C_{18}H_{22}O_4$ Carbon = 71.52% Hydrogen = 7.28%
Found Carbon = 70.92% Hydrogen = 7.02%

α-Naphthyl chrysanthemumate—Procedure C.
M. p. 53°
Calculated for $C_{20}H_{22}O_2$ Carbon = 81.63% Hydrogen = 7.48%
Found Carbon = 81.57% Hydrogen = 7.23%
" 81.40% " 7.52%

SUMMARY

1. Various esters of chrysanthemum monocarboxylic acid (2,2,dimethyl-3-isobutylene-trimethylene-1-carboxylic acid) have been prepared and their refractive indices have been determined.

2. The lauryl, myristyl, cetyl, and diethanolamine esters at a concentration of 0.03 per cent showed a kill of 60.4, 62.0, 65.3, and 63.6 per cent toward *Aphis rumicis* as compared to a kill of 70 per cent for the pyrethrins at the same concentration.

3. None of the esters produced the typical pyrethrin action when applied to various parts of the cockroach (*Periplaneta americana* L.)

4. Except for the furfuryl and vanillin esters, the compounds prepared showed no signs of decomposition or loss of toxicity after six months. This suggests that the instability of the pyrethrins is due to the ketonic alcohol, pyrethrolone.

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FACTORS INFLUENCING SUSCEPTIBILITY OF PLANTS TO SULPHUR DIOXIDE INJURY. I

CARL SETTERSTROM AND P. W. ZIMMERMAN

For more than ninety years the effects of sulphur dioxide on vegetation have received the attention of various agricultural and industrial interests, and considerable data have accumulated on many phases of this subject. It is the purpose of the present paper to provide some basis for evaluating these many data and to afford a broader view of the mechanism of sulphur dioxide injury. The influence of a number of environmental factors on susceptibility of plants to sulphur dioxide injury is reviewed, and considerable new data are added to those phases of the problem which are treated less extensively in the literature. The new data have been obtained from factorial experiments designed to study a number of variables simultaneously, and all the data have been subjected to statistical analysis by the method of the analysis of variance.

The environmental factors considered include temperature, humidity, soil moisture, soil fertility, nutrient supply, pretreatment with sulphur dioxide, light intensity, age of plants, and moist surfaces. The literature and new data are discussed together under the heading of the relevant influencing factor.

DESIGN OF EXPERIMENTS

The design of the fumigation experiments for statistical treatment was similar to that discussed in a previous paper (17). The experiments were of a factorial type, a number of factors being studied simultaneously. This scheme permits the mobilization of the entire experiment in support of each contrast. Improvement in precision results from the large number of plants involved, and greater dependence may be placed on the result because the contrast rests on a broad basis of varied conditions rather than on some arbitrary standard running through the whole group. Factorial experiments of this nature are especially adapted to determining whether the effect of a given factor is more pronounced under some conditions than under others.

The final treatment in each of the experiments consisted of a sulphur dioxide fumigation severe enough to cause considerable injury to the most susceptible of the plants but not severe enough to cause more than slight markings on the most resistant plants. After the markings caused by this injurious treatment had become readily visible, the plants, which had been subjected to varying pretreatments, were arranged according to intensity of visible markings. In most cases, all the plants in one pot were given the same pretreatment and the pots rated as units. Those engaged in rating the plants were not cognizant of pretreatments that had been given to any particular plant or pot.

Instead of the usual tedious estimation of the per cent leaf area injured, the pots were numbered consecutively beginning with the least injured as number one. The resultant data were recorded and treated statistically according to the method of the analysis of variance (3). In interpreting the analyses of variance, differences which could have occurred by chance not more than once in 20 trials are considered significant; differences which could have occurred by chance not more than once in 100 trials are considered highly significant.

TABLE I

COMPARATIVE RATINGS OF POTS OF BUCKWHEAT; EXPERIMENT 27; LEAST INJURED RATED 1

Variables				Replicates				Totals	Order of totals
Rating by E.B.	Ethylene pretreatment	Good soil	Ample water	10	11	21	22	64	5
			Insufficient water	1	2	3	5	11	8
		Poor soil	Ample water	15	17	25	29	86	3
			Insufficient water	23	24	28	31	106	2
	No ethylene pretreatment	Good soil	Ample water	13	16	18	20	67	4
			Insufficient water	6	7	8	9	30	7
		Poor soil	Ample water	26	27	30	32	115	1
			Insufficient water	4	12	14	19	49	6
Rating by C.S.	Ethylene pretreatment	Good soil	Ample water	10	11	15	17	53	5
			Insufficient water	1	2	3	5	11	8
		Poor soil	Ample water	23	24	26	31	104	2
			Insufficient water	16	21	25	27	89	3
	No ethylene pretreatment	Good soil	Ample water	13	18	20	22	73	4
			Insufficient water	4	6	8	9	27	7
		Poor soil	Ample water	28	29	30	32	119	1
			Insufficient water	7	12	14	19	52	6

In order to determine the precision of the method of rating described, the plants in experiment 27 were rated entirely independently by two observers. The data are given in Table I. All the factorial experiments are summarized in Table II. At the suggestion of W. J. Youden of this Institute, the ratings in several of the experiments were corrected by using the scores of Fisher and Yates' Table XX (4, p. 50) which gives the average deviate of each sample number of a particular number of observations drawn from a normal distribution having unit variance. The scores were

TABLE II
SUMMARY OF FACTORIAL EXPERIMENTS

Exp. No.	Variables, in order of significance	Most susceptible plants or treatment resulting in greatest injury	Significant interactions, in order of significance	No. of pots used	Age of plants, days	Av. SO_2 concn., p.p.m.	Av. rel. humid., %	Av. temp., ° F.	Time of fumigation	Date of fumigation
1	Nutrient supply** SO_2 pretreatment Sulphur content Water supply—fg	Deficient nutrients	None	48	43	0.87	45	91	8:40 A.M.– 1:40 P.M.	May 25
2	Water supply—fg** Sulphur content SO_2 pretreatment	Ample water supply	None	48	21	0.95	50	80	1:53 P.M.– 6:53 P.M.	May 27
3a	Water supply—fg** Dark pretreatment Sulphur content Nutrient supply	Ample water supply	None	48	48	1.06	45	109	10:19 A.M.– 2:19 P.M.	June 7
3b	Water supply—fg* Sulphur content	Ample water supply	None	12	18	1.06	45	109	10:19 A.M.– 2:19 P.M.	June 7
4a	Water supply—g** Sulphur content SO_2 pretreatment	Ample water supply	Water vs. sulphur*	24	33	0.91	47	83	8:59 A.M.– 2:29 P.M.	June 22
4b	Water supply—g** SO_2 pretreatment** Nutrient supply* Sulphur content	Ample water supply (see text p. 172) Deficient nutrients	None	36	66	0.91	47	83	8:59 A.M.– 2:29 P.M.	June 22
4c	Shade pretreatment* Sulphur content Water supply	Heavy shade	None	12	17	0.91	47	83	8:59 A.M.– 2:29 P.M.	June 22
5	Age of plants** Water supply—f Shade pretreatment	Older plants	None	36	28 234	1.12	45	81	9:00 A.M.– 2:30 P.M.	June 27

TABLE II (*Continued*)

Exp. No.	Variables, in order of significance	Most susceptible plants or treatment resulting in greatest injury	Significant interactions, in order of significance	No. of pots used	Age of plants, days	Av. SO_2 concn., p.p.m.	Av. rel. humid., %	Av. temp., ° F.	Time of fumigation	Date of fumigation
6	Water supply—fg** Shade pretreatment**	Ample water supply Heavy shade	None	24	20	1.20	61	59	2:30 P.M.– 6:30 P.M.	June 27
7	Age of plants** Light quality** Water supply—f	Oldest plants Red light	None	56	43 160 226 273	1.54	56	66	10:20 A.M.–10:24 P.M. 11:45 A.M.– 1:59 P.M.	Oct. 20 Oct. 21
8	Water supply—g** Water supply—f** Light quality**	Ample water supply Ample water supply Red light	Water—g vs. light,** Water—g vs. water—f vs. light*	100	19	1.06	64	73	5:30 A.M.–10:30 A.M.	May 25
9	Water supply—g** Soil fertility**	Ample water supply Poor soil	None	48	21	1.26	49	81	7:15 A.M.–12:15 P.M.	May 27
10	Water supply—g** Light quality**	Ample water supply White light	None	40	26	1.12	39	86	7:10 A.M.– 1:10 P.M.	June 1
11	Water supply—g** Soil fertility** Shade pretreatment*	Ample water supply Poor soil Heavy shade	Water vs. shade** Soil vs. shade*	96	27	1.16	41	92	6:53 A.M.– 2:55 P.M.	June 2
12	Water supply—g** Soil fertility** Moist surface	Ample water supply Poor soil	Water vs. surface,** Water vs. soil,** Water vs. soil vs. surface**	56	32	1.34	43	97	8:22 A.M.–12:22 P.M.	June 7
13	Soil fertility** Dark pretreatment**	Poor soil No dark pretreatment	Soil vs. dark**	72	33	1.32	23	98	1:13 P.M.– 4:18 P.M.	June 8
14	Water supply—g** Light quality**	Ample water supply Blue light	Water vs. light*	60	10	1.16	51	86	6:58 A.M.–11:58 A.M.	June 21

TABLE II (*Continued*)

Exp. No.	Variables, in order of significance	Most susceptible plants or treatment resulting in greatest injury	Significant interactions, in order of significance	No. of pots used	Age of plants, days	Av. SO_2 concn., p.p.m.	Av. rel. humid., %	Av. temp., ° F.	Time of fumigation	Date of fumigation
15	Water supply—g** Shade pretreatment** Soil fertility	Ample water supply Heavy shade	None	96	15	0.89	57	93	12:02 P.M.– 5:02 P.M.	June 21
16a	Water supply—g** Light quality** Moist surface	Ample water supply Blue light	Water vs. light**	60	12	1.06	57	82	7:13 A.M.–12:13 P.M.–	June 23
16b	Water supply—fg** Soil fertility Moist surface	Ample water supply	None	16	9	1.06	57	87	7:13 A.M.–12:13 P.M.	June 23
17	Age of plants** Water supply—g** Soil fertility*	Oldest plants Ample water supply Poor soil	Age vs. water** Age vs. soil*	72	5 11 19	1.09	53	92	7:04 A.M.–12:04 P.M.	June 25
18	Water supply—g** Light quality**	Ample water supply Red light	Water vs. light**	60	16	1.36	64	66	8:07 A.M.– 1:07 P.M.	June 27
19	Water supply—g** Soil fertility** Shade pretreatment**	Ample water supply Poor soil Heavy shade	Water vs. shade** Soil vs. shade*	96	21	1.20	68	65	9:38 A.M.– 2:38 P.M.	June 27
20	Soil fertility** Water supply—f** Dark pretreatment	Poor soil Ample water supply	None	72	8	1.76	52	71	12:16 P.M.– 7:16 P.M.	June 28
21	Age of plants** Water supply—g** Soil fertility**	Oldest plants Ample water supply Poor soil	Age vs. water** Age vs. water vs. soil*	72	9 15 23	1.17	37	95	10:53 A.M.– 3:53 P.M.	June 29
22	Soil fertility** Water supply—g** Time of day**	Poor soil Ample water supply Morning	Soil vs. water* Soil vs. time*	96	8	2.45	47	93	5:00 A.M.–11:00 A.M. 11:00 A.M.– 5:00 P.M. 5:00 P.M.–11:00 P.M. 11:00 P.M.– 5:00 A.M.	June 28 to June 29

TABLE II (*Continued*)

Exp. No.	Variables, in order of significance	Most susceptible plants or treatment resulting in greatest injury	Significant interactions, in order of significance	No. of pots used	Age of plants, days	Av. SO_2 concn., p.p.m.	Av. rel. humid., %	Av. temp., ° F.	Time of fumigation	Date of fumigation
23	Water supply—g** Dark pretreatment** Soil fertility**	Ample water supply Three days in dark Poor soil	None	96	10	1.29	39	100	9:30 A.M.– 3:30 P.M.	June 30
24	Soil fertility** Water supply—g** Shade pretreatment**	Poor soil Ample water supply Heavy shade	Soil vs. water**	96	25	1.05	66	77	7:18 A.M.–11:32 A.M.	July 1
25	Quality of light** Water supply—g	Blue light	None	60	20	1.32	59	78	7:20 A.M.–11:33 A.M.	July 1
26	Age of plants** Water supply—g** Soil fertility**	Oldest plants Ample water supply Poor soil	None	72	11 17 25	1.42	53	80	12:50 P.M.– 6:50 P.M.	July 1
27	Water supply—g** Dark pretreatment** Soil fertility**	Ample water supply No dark pretreatment Poor soil	Water vs. soil**	72	12	5.34	48	87	9:03 A.M.–10:45 A.M.	July 2
28	Soil fertility** Water supply—g** Ethylene	Poor soil Ample water supply	Soil vs. ethylene,** Water vs. ethylene**	32	18	1.15	58	84	7:25 A.M.–10:00 A.M.	Aug. 20
29	Age of plants** Water supply** Soil fertility** Moist surfaces*	Older plants Ample water supply Poor soil Wet surfaces	Age vs. soil** Age vs. water** Water vs. soil*	48	22 38	1.09	43	79	8:37 A.M.–12:37 P.M.	Sept. 9

Note: The term "water supply—f" is used to designate differences in water supply during fumigation; "water supply—g" is used to designate differences in water supply during growth, prior to fumigation; "water supply—fg" indicates differences during both fumigation and growth. Alfalfa was used in experiments 1, 3a, 3b, 4a, 4b, and 5. Buckwheat was the test plant in all the other experiments.

* Significant.

** Highly significant.

subjected to analyses of variance and the results compared with the analyses of the original rating data. In none of the experiments recalculated was there any difference in the results. To avoid the extensive and apparently unnecessary labor of recalculating all the data, the analyses of the original ranking data have been used in this paper. The use of the Fisher and Yates scores, however, is recommended as the more appropriate treatment.

APPARATUS

The apparatus employed for the sulphur dioxide fumigations consists of duplicate sets. Each set includes a cabinet of special design and construction in which the plants are placed during the experiment; a variable speed exhauster-blower and an orifice meter for precise control of the volume of air passing through the cabinet; an especially designed scrubber attached to the intake side of the blower to rid the air of SO_2 before it enters the system and to effect partial control of the humidity in the cabinet; a Bristol humidigraph for recording the temperature and relative humidity in the cabinet; a Thomas autometer; and a Leeds and Northrup conductivity recorder for recording sulphur dioxide concentrations in the cabinet.

All the apparatus, except the conductivity recorders, is housed in a small greenhouse equipped with automatic temperature control. The conductivity recorders are installed in a laboratory about 20 feet from the greenhouse.

Accurate metering of the sulphur dioxide entering a cabinet is made possible by the use of calibrated capillary flowmeters, a reducing regulator, a sensitive needle valve, and a cylinder containing a special mixture of 5 per cent sulphur dioxide and 95 per cent air.

In each experimental fumigation the concentrations of SO_2 were established by means of a calibrated capillary flowmeter and a calibrated orifice meter, and were finally determined with the autometer-recorder system. In all cases the calculated and observed values were in agreement.

For a detailed description of the apparatus see a previous paper by Setterstrom and Zimmerman (16).

EXPERIMENTAL PROCEDURE AND RESULTS

In order to illustrate the procedure employed in the 29 factorial experiments used as a basis for evaluating the influence of the various environmental factors, one experiment, number 23, will be described in detail.

Soil fertility, water supply, and dark pretreatments were the factors studied in the illustrative experiment. Buckwheat (*Fagopyrum esculentum* Moench.) was selected as the test plant because it is sensitive to sulphur dioxide injury, and its large leaves permit more rapid and accurate rating of the degree of injury than is possible with alfalfa or similar sensitive plants.

The buckwheat, planted June 20, 1938 and grown from seed in five-inch pots, averaged about nine plants per pot. Half of the plants were grown in a composted soil, the other half in a mixture of one part of poor soil and one part of sand. Half of the plants of each soil treatment were grown with an ample supply of water, the other half with just enough water to keep the plants from wilting. The success of these soil and water treatments in producing plants in varying stages of development is illustrated in Figure 1. A set of 24 pots, six pots of each of the four combinations of soil and water treatments, was placed in absolute darkness for three days just prior to the SO_2 treatment, a like set was kept in the dark two days, another for one day, and one set was given no dark treatment. The various pots were assigned to the differing treatments solely by chance.

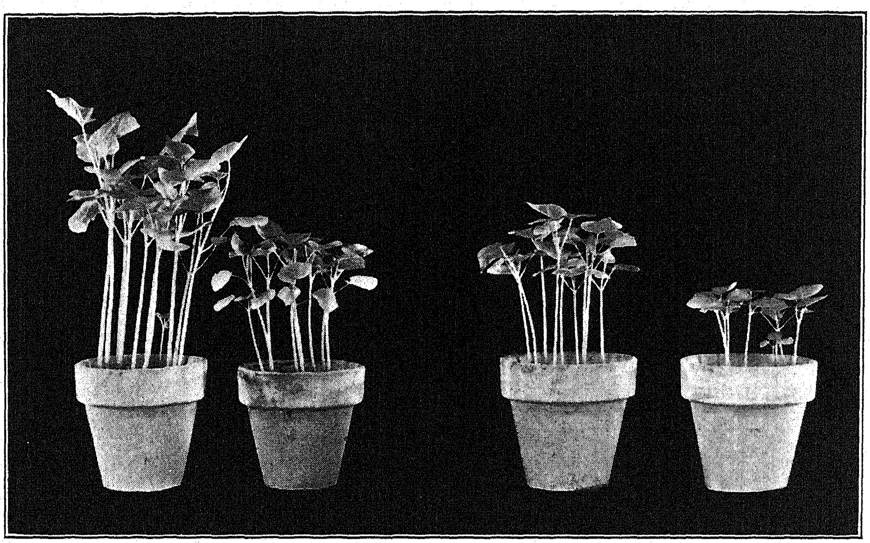

FIGURE 1. Illustrating the success of the treatments of water supply and of soil fertility designed to produce a wide range of plant development for a study of comparative susceptibility to sulphur dioxide injury. The buckwheat was planted June 6, photographed June 21. Left to right: Ample water, good soil; insufficient water, good soil; ample water, poor soil; insufficient water, poor soil.

The plants were treated with sulphur dioxide when 10 days old. They had germinated quickly, grown rapidly and were three to five inches high, depending upon the pretreatments at the time of the sulphur dioxide fumigation. Plants given the same pretreatments were uniform.

The plants were treated with sulphur dioxide from 10:30 A.M. to 4:30 P.M. on June 30, 1938. The inlet concentration of sulphur dioxide as calculated from capillary flowmeter and orifice meter reading was 1.40 parts of sulphur dioxide per million parts of air (p.p.m.). The average inlet concentration as determined by the recorder readings was 1.36 p.p.m.; the maximum reading was 1.49 p.p.m.; the minimum 1.21 p.p.m. The average outlet concentration as determined by the recorder readings was 1.22 p.p.m.; the maximum was 1.39 p.p.m.; the minimum 1.10 p.p.m. The volume of air passing into the cabinet was 158 cubic feet per minute.

The average temperature inside the cabinet, determined by averaging quarter-hour humidigraph readings, was 100.1° F. The maximum temperature was 105°; the minimum 95°. Average relative humidity was 39 per cent; maximum 53 per cent; minimum 33 per cent. The sky was partly cloudy at 10:30 A.M., began clearing at 11:00 A.M. and was clear from 11:15 A.M. to 4:30 P.M.

Immediately before the SO_2 treatment, all the pots were watered uniformly, then placed in saucers filled with water. The saucers were refilled about 11:30 A.M. and again after the fumigation was discontinued.

By the next day the flaccid, sulphur dioxide marked areas had bleached to a readily discernible yellow-white; so the pots were arranged in line on the greenhouse floor in order of degree of injury, the arrangement being checked by two workers. The pots then were numbered progressively beginning with the least injured. The first nine had no visible markings, so all nine were rated five, and consecutive numbering began with pot ten. The rating sheet is given in Table III together with tables grouping the effects of the several variables. These data then were subjected to statistical treatment by the method of the analysis of variance and the results are shown in Table IV.

Examination of the analysis of variance reveals that the effect of each of the three variables is highly significant, and that the effect of the water treatments is greater than that of the dark treatments which in turn is greater than the effect of the soil treatments. None of the interactions is significant indicating that the effects of the several variables were the same under all the conditions studied.

Similar data were prepared for each of the other 28 experimental fumigations, but limitations of space prevent publication of the numerous resultant tables. All the factorial experiments are summarized in Table II. The general plan of each of the experiments is similar to the one described; differences and specific details will be referred to under the various sections which follow.

Buckwheat (*Fagopyrum esculentum* Moench.) was selected as the test plant in most of the experiments, as has already been noted, because of its sensitivity and because its large leaves permit rapid and accurate rating of degree of injury. Alfalfa (*Medicago sativa* L. var. Grimm), also sensitive to sulphur dioxide injury and economically important in certain areas subjected to sulphur dioxide fumes, was used in certain cases.

TABLE III

RATING OF POTS OF BUCKWHEAT; EXPERIMENT 23

Variables			Replicates						Totals
3 days dark pretreatment	Good soil	Ample water	51	72	75	79	85	86	448
		Insufficient water	41	57	61	64	69	93	385
	Poor soil	Ample water	84	89	90	91	92	96	542
		Insufficient water	24	34	35	42	68	95	298
2 days dark pretreatment	Good soil	Ample water	37	40	52	65	66	81	341
		Insufficient water	10	21	29	33	36	78	207
	Poor soil	Ample water	59	67	82	83	87	88	466
		Insufficient water	19	20	30	55	76	80	280
1 day dark pretreatment	Good soil	Ample water	43	49	56	58	60	71	337
		Insufficient water	5	5	16	32	45	74	177
	Poor soil	Ample water	46	63	70	73	77	94	423
		Insufficient water	22	44	47	48	54	62	277
No dark treatment	Good soil	Ample water	15	17	26	28	31	38	155
		Insufficient water	5	5	5	5	11	18	49
	Poor soil	Ample water	23	25	27	39	50	53	217
		Insufficient water	5	5	5	12	13	14	54

Recapitulation

	Good soil	Poor soil	Total
Ample water	1281	1648	2929
Insufficient water	818	909	1727
Total	2099	2557	4656

	Days in dark				Total
	3	2	1	0	
Good soil	833	548	514	204	2099
Poor soil	840	746	700	271	2557
Total	1673	1294	1214	475	4656

	Days in dark				Total
	3	2	1	0	
Ample water	990	807	760	372	2929
Insufficient water	683	487	454	103	1727
Total	1673	1294	1214	475	4656

While some of the discussion and conclusions which follow are stated rather broadly as applicable to plants in general, it should be noted that buckwheat and alfalfa were the only plants used in the experiments of the present series, and that it is quite possible that susceptibility of all plants will not be similarly influenced by the various factors.

Light intensities at times of fumigations, and other meteorological data, have not been given in any detail because such data, if desired, can be obtained from reports (13) of the New York City Meteorological Observatory. Experiments 8, 10, 19, 20, 21, 29, and 30 were performed when the sky was cloudy, experiments 4, 9, 17, 18, 26, 28, and 31 when the sky was partly cloudy, and all other experiments when the sky was clear. The various experiments extend over a period of two calendar years. Experiments 1 to 8, inclusive, were performed in 1937, the others in 1938.

TABLE IV

ANALYSIS OF VARIANCE OF DATA IN TABLE III; EXPERIMENT 23

Source of variation	Degrees of freedom	Sum of squares	Mean square	F†
Water supply	1	15050	15050	54.0**
Dark pretreatment	3	31383	10461	37.6**
Soil fertility	1	2185	2185	7.9**
Water vs. soil	1	794	794	2.9
Dark vs. soil	3	1080	360	1.3
Water vs. dark	3	61	20	0.1
Water vs. dark vs. soil	3	826	275	1.0
Replicates	80	22281	279	
Totals	95	73660		

† F is a symbol used by Snedecor (18) to designate the ratio of the larger mean square to the smaller. The significance of F is determined by consulting Snedecor's Table XXXV.

** Highly significant.

TEMPERATURE

Swain (20, p. 298) concludes, "A plant is much more resistant to sulphur dioxide at temperatures of 5° C. and below than it is at higher temperatures, yet, there is no considerable variation above 5° C. within the usual range of atmospheric temperatures." The critical temperature of 5° C. is in close agreement with the value of 40° F. suggested by Wells (25) who bases his conclusions on extensive experimental data compiled by O'Gara for American Smelting and Refining Company.

Stoklasa (19, p. 57) sets no critical temperature, but argues that the higher the temperature, the more toxic should be the gas. Katz and Ledingham (11, p. 16) state that at high temperatures, i.e., above 100° F., the plants become increasingly resistant to SO_2.

In the present study, experiments on temperature effect have been incidental to studies of transpiration rate which will be discussed in a subsequent paper. In these experiments buckwheat, 25 to 30 days old, grown in good soil with an ample water supply was equally susceptible to SO_2 injury at 65° and at 105° F. under similar conditions of humidity and light intensity.

HUMIDITY

In 1903, Widstoe (26, p. 166) reported "It is a well established fact that in a moist atmosphere or with moistened plants the injury due to sulphur dioxide is much more severe than under dry conditions," but in the same year Haselhoff and Lindau (6) declared that the absorption of sulphurous acid—and accordingly the disturbance in water circulation—is greater in the light, in high temperature, and dry air, than in darkness, low temperature, and moist air.

Ebaugh (2) disagreeing with Haselhoff and Lindau maintained that injury usually occurred in damp weather rather than during bright, clear, dry weather. Observations of Wislicenus (28) and Stoklasa (19) supported those of Widstoe and Ebaugh.

Experimental data obtained by Wells while working for the Selby Smelter Commission (8) support the observations of Widstoe *et al.* "It was conclusively shown by the investigation [of Wells] that the quantity of water vapor present in the atmosphere (humidity) is a strong determinative factor in the production of injury. Given concentrations in a dry atmosphere for the same period of time did not produce as extensive injury as did the same concentrations in a moist atmosphere, although the work was not extended sufficiently in this direction to establish a relationship of quantitative nature relative to humidity" (8, p. 43).

O'Gara attempted to establish the effects of humidity on a quantitative basis and reported (14, p. 11) that at 30 per cent relative humidity plants are three times as resistant to sulphur dioxide as they are at 100 per cent. Wells (25, p. 642) reviewing O'Gara's work declares "In the Salt Lake Valley it was found that 70 per cent was about the critical humidity; above that point plants were much more susceptible to sulphur dioxide than below." Swain (20, p. 298) in a later review reports, "There is not much increase in sensitiveness from 70 per cent relative humidity to 100 per cent, but the resistance of a plant increases more rapidly when this drops below 70 per cent and becomes very prominent below 50 per cent."

Zimmerman and Crocker (30) found that a difference of 20 per cent relative humidity, in a range between 50 and 75 per cent, did not cause the plants to become either more or less resistant. Katz and Ledingham (12, p. 20) while admitting "it is rather difficult to secure duplication of results with similar SO_2 treatments" declare that in general resistance decreases with increase in relative humidity provided other factors remain constant. They cite experiments with alfalfa in which treatment with 0.75 p.p.m. of SO_2 caused injury in two and one-quarter hours when the humidity was over 80 per cent, in five hours when the humidity was from 60 to 80 per cent, and in eleven hours at 40 to 60 per cent humidity.

Fisher and Goldsworthy (23) conclude (p. 336) from their experiments that the effect of sulphur dioxide on alfalfa was not influenced to a great extent by the relative humidity used (40 to 70 per cent), but they later (p. 337) modify their conclusion with the statement, "plants are more likely to be injured to a greater degree as the relative humidity of the environment increases."

Thomas and Hill (21, p. 294), as a result of plotting leaf area of alfalfa destroyed by SO_2 treatment against the amount of gas absorbed, conclude that a given quantity of absorbed gas is only slightly less effective, if at all, in destroying leaf tissue at humidities below 80 per cent than above 80 per cent. This indicates that humidity affects rate of absorption rather than susceptibility of the plant.

Several explanations for the effect of humidity on susceptibility have been advanced. Swain (20), O'Gara (14, p. 11), and Katz and Ledingham (12) agree essentially with Wells (25, p. 642) that low humidity is very effective in reducing the turgidity of the guard cells and thus causing closure of the stomata with resultant decrease in SO_2 absorption.

Haselhoff and Lindau (6), however, maintain that the stomata of leaves play no part in the absorption of SO_2, that the gas is, in fact, not taken up through the stomata but by the entire leaf surface. Therefore, they reason, the amount of SO_2 penetrating the leaf is not conditioned by the number or by the condition of the stomata, but by other factors in the individual organism. Von Schroeder and Schmitz-Dumont (15) and Yonemaru (29) hold similar views. Janson (9) maintains that gas enters only through the stomata and that the cuticle is insensitive to the gas. Verplancke (24), while agreeing that the gas penetrates the tissue by way of the whole foliar surface, suggests that the stomata allow the SO_2 to pass when they are open under the combined action of light and humidity.

Yonemaru explains that sulphur dioxide is more injurious in wet than in dry weather because the gas is changed into fine drops through absorption of water, thereby becoming more concentrated and on account of the consequent slow motility, remaining longer in contact with the plant.

Fisher and Goldsworthy (23, p. 337) suggest that as the relative humidity increases "the increase in the water content of the leaves may permit a greater absorption of SO_2 in the cells of the mesophyll or spongy leaf tissue below the stomatal openings."

All the data on the influence of humidity have treated the effect of varying humidity at time of treatment with sulphur dioxide. In the light of studies on the effect of soil moisture which will be discussed later, it seems likely that studies of the effect of growth of plants in various humidities prior to SO_2 treatment might prove of interest.

The results of experiment 4 suggest that plants grown in atmospheres of higher humidity are more susceptible to subsequent SO_2 injury. Alfalfa pretreated with 1.00 p.p.m. of SO_2 for four hours was significantly less injured than were alfalfa which was pretreated with 0.19 p.p.m. for 118 hours and alfalfa which was not exposed to SO_2 pretreatment. This explanation is offered: After pretreatment the "1.00 p.p.m. alfalfa" was placed on the greenhouse benches while the other alfalfa was kept in the SO_2 and control fumigation cabinets at higher humidities than prevailed in the greenhouse.

SOIL MOISTURE

Katz (10, p. 18) has pointed out that the effect of high soil moisture is similar to that of high relative humidity in increasing the susceptibility of plants to sulphur dioxide. Katz suggests that "the main factor in each case is the condition of the stomata. When the soil moisture is very low or the relative humidity of the atmosphere is low, the leaf stomata tend to close in order to guard against excessive transpiration losses. At such times there can be very little absorption of sulphur dioxide except by diffusion through the cuticles."

Zimmerman and Crocker (30), noting that wilted plants were more resistant than similar plants that were turgid, considered the difference to be due, at least in part, to the condition of the stomata. Wislicenus and associates (28) use the fact that dry plants are more resistant than moist plants to illustrate that gases do not penetrate when stomata are closed.

In a series of experiments in which temperature, humidity, gas concentration and other factors were maintained as closely similar as possible, the only variable being soil moisture, Katz (11, p. 56), and Katz and Ledingham (12, p. 24) showed that when the plants approached the wilting point there was a marked decrease in susceptibility, although minor variations in soil moisture, which was adequate for growth, were without effect.

In the present study, two phases of the influence of soil moisture on susceptibility have been considered; soil moisture at time of fumigation, and soil moisture during growth prior to fumigation.

No attempt was made to determine moisture content of the soil quantitatively as was done by Katz and Ledingham, but qualitatively, at least, the results of experiments on soil moisture at time of fumigation support the conclusions of Katz and Ledingham, and of Zimmerman and Crocker: Plants at or near the wilting point are more resistant than plants with an ample moisture supply; minor variations in moisture content are without effect.

In experiment 5 (using 36 pots of alfalfa) and experiment 7 (using 56 pots of buckwheat), half the plants were given an ample water supply—they were well-watered before the sulphur dioxide treatment and kept in water-filled saucers during the fumigation. The other half were kept relatively dry—they were watered lightly before the fumigation and given no further water during the treatment. None of the plants showed any symptoms of wilting, and in none of these experiments did the differences in water supply at time of fumigation have any effect on the susceptibility of the plants to sulphur dioxide injury.

In experiments 9 and 23, however, using 100 and 72 pots of buckwheat respectively, the differences in water supply during treatment resulted in highly significant differences in SO_2 susceptibility, those plants getting more water were injured to a greater degree. In both of these experiments half of the plants were well-watered, as described above. The dry pots had received no water in over 24 hours and were near wilting but did not actually begin to wilt during the sulphur dioxide treatment. In experiment 9, water supply during growth prior to treatment had a greater effect on susceptibility than did water supply during treatment, and in experiment 23 the quality of light was of greater significance.

Other variables in these "water supply during fumigation" experiments were age of plants, shade pretreatment, quality of light, water supply during growth, soil fertility, and dark pretreatment, but in none of the experiments was there a significant interaction with water supply during fumigation.

No data have been uncovered in the literature on the effect of soil moisture during growth as distinct from soil moisture during the fumigation, although Janson (8) and other "smoke injury experts" believe that well-irrigated plants are much more resistant to injury than poorly-irrigated plants.

Actually, however, the well-irrigated plants have been found by us to be much more susceptible to injury than plants grown with a deficient water supply. In all but two of the twenty-four factorial esperiments in which soil moisture during growth was a variable, the effect on sulphur dioxide susceptibility was of great significance. In all of the 22 experiments in which the effect was significant, the odds against the differences having occurred by chance were greater than 1000 to 1. A total of 60 pots of alfalfa and 1428 pots of buckwheat were used in these 24 experiments; and other variables involved include sulphur content of nutrient supply, sulphur dioxide pretreatment, nutrient supply, water supply during fumigation, quality of light, soil fertility, shade pretreatment, moist surfaces, dark pretreatment, time of day, age of plants, and ethylene pretreatment.

In each of the 23 experiments of the soil moisture series, half the plants were grown with an ample supply of water, the other half were grown with just enough water to keep the plants from wilting. The differences in water supply were initiated as soon as the seeds had germinated (all the pots were kept moist until this time to insure uniform germination) and maintained until several hours just prior to fumigation, when uniform watering was begun. During fumigation all the plants were placed in saucers filled with water to insure a uniform supply of water to the various pots. In the two experiments (4 c and 25) in which the effect of growth-water was not significant the plants were quite young, and it is believed that the differences in water treatment were not of sufficient duration to condition susceptibility significantly. In these two experiments there was no visible difference between the "wet" and "dry" plants at time of fumigation. Differences in water supply during growth usually brought about marked differences in the appearance of the plants. The plants grown with an ample water supply were taller and more succulent than the plants grown with a deficient water supply, as can be seen in Figure 1. The older the plants, the greater were the differences in appearance.

In six experiments, using in all 96 pots of alfalfa and 56 pots of buckwheat, half of the plants were grown with a deficient water supply and kept dry during the fumigation. The other half were grown with ample water and kept well-watered during the fumigation. In every case but one, experiment 2 b, the effect of water supply was additive and the increased susceptibility of the well-watered plants was highly significant. In experiment 2 b, although the "dry" plants were not given as much water as the well-watered, the difference in water treatments was not as great as in the other experiments. The increased susceptibility of the well-watered plants, therefore, was not marked enough to be significant when only 24 pots were used.

Of all the variables studied, only the age of the plants had a more marked effect on susceptibility than did the supply of water during growth. Soil fertility or nutrient supply, however, had a greater effect in three of the seventeen experiments in which these two variables were considered together. The various significant interactions in which soil moisture is one variable will be considered under the headings of the other variables.

This factor of soil moisture assumes practical significance in applying results of fumigation experiments on well-irrigated experimental plots to field crops in an arid or poorly-irrigated area. Though it has been clearly shown that this factor is of great significance, sufficient data have not been accumulated to permit quantitative evaluation of its effect on susceptibility.

NUTRIENT SUPPLY AND SOIL FERTILITY

The influence of soil fertility on the susceptibility of plants to sulphur dioxide has attracted little attention in the literature, although the question of susceptibility of poorly growing plants has long troubled smoke injury appraisers who have had to base their findings on experimental fumigations of plants growing under optimum conditions.

Stoklasa (19, p. 56), among others, is of the opinion that the toxic action of sulphurous acid in the plant organism proceeds the more intensively when the root systems of the plants are inadequately supplied with organic nutrients for the formation of new plant substance, but the only experimental work relating to this phase of the problem seems to be that of Wells (25) who found no material difference in duplicate experimental fumigations performed on barley growing in a poorly cultivated field and in a well cultivated field. Admittedly, however, only gross differences could have been detected in these experiments.

The present studies on this phase of the problem have been divided into two classes which are termed nutrient supply and soil fertility. "Nutrient supply" is used as the notation in those experiments in which the plants were grown in sand and watered with nutrient solutions prepared as described in a previous paper (17, p. 182). Half of the plants were given the so-called normal supply of nutrients twice a week, the other half were given one-tenth the normal supply once or twice a week. Nutrient supply was a variable in three factorial experiments; in one its effect on susceptibility was not significant, in another its effect was significant, and in the third its effect was highly significant. In each of these experiments alfalfa was the test plant and the plants with a deficient nutrient supply proved more susceptible. The alfalfa plants grown with a deficient nutrient supply were smaller and noticeably more yellow in each case, but the greater the difference in appearance between the two sets of alfalfa, the greater was the difference in susceptibility.

The term "soil fertility" is used as a designation in the experiments in which the plants were grown in soils of differing fertility. In the 17 factorial experiments (using in all 1216 pots of buckwheat) in which soil fertility was a variable, half of the plants were grown in a good compost, the other half in a mixture of one part sand to one part of poor soil. In 14 of the 17 experiments, the greater injury to the plants grown in the poorer soil was found to be highly significant, and in one experiment the difference was just significant, while in two experiments the difference was not large enough to be significant.

As is shown in Figure 1, the plants grown in poor soil were smaller than the corresponding plants grown in good soil. Here, again, as was the case in connection with other variants, the more marked the difference in appearance, the greater was the difference in susceptibility.

Except for the effects of water supply during growth, and of the age of the plants, the effect of the soil fertility variations was more significant than that of any of the variables studied. Proper evaluation of this factor, as well as that of water supply, is necessary before the results of experimental fumigations performed under optimum conditions can be applied to field conditions.

In five of seventeen experiments, the interaction between soil and water was significant because of the greater effect of soil fertility on the well-watered plants. These significant interactions are believed due to the different moisture-retaining capacities of the types of soil used. Other significant interactions in which soil moisture is one variable will be considered under the headings of the other variables.

SULPHUR CONTENT OF NUTRIENT SUPPLY

It is generally recognized that exposure to sulphur dioxide is one means of bringing about an increase in sulphur content of plants, and Thomas and Hill (21) have successfully used sulphur determinations quantitatively to check their data on rate of absorption of sulphur dioxide. Certain supporters (22) of the invisible injury theory (17) advance this increase in sulphur content as proof of invisible injury, holding that each exposure to sulphur dioxide increases the sulphur content of the plants with resultant greater sensitivity to injury by the gas.

In order to test the validity of the assumption that increased sulphur content denotes increased susceptibility, a series of experiments have been performed with plants of varying sulphur content.

In order to obtain material with varying sulphur content the plants used in this series of experiments were grown from seed in five-inch pots containing sand and were watered once or twice weekly with nutrient solutions containing varying amounts of sulphur in the form of sulphate. The method of preparing these solutions has been described in a previous paper (17, p. 183). In experiments 1, 2, and 3 a, the amounts of sulphur in the nutrient solutions were set at 0.083 per cent, 0.033 per cent, and 0.013 per cent. In experiments 3 b, 4 a, 4 b, and 4 c, the amounts of sulphur were more widely varied at 0.165 per cent, 0.033 per cent, and 0.007 per cent. These sulphur percentages were established by adding varying amounts of K_2SO_4, $MgSO_4$, and $MgCl_2$ to the nutrient solutions in such ratios that the percentage of magnesium ions in solution was the same in each case. The percentage 0.033 was designated normal supply of sulphur because it was found to be the optimum amount for highest yields.

In none of the experiments of this series was the sulphur content of the plants grown in the various nutrient solutions determined, but previously reported sulphur determinations (17, p. 193) of alfalfa grown under similar conditions furnish evidence that differences in sulphur content of the plants do result.

In none of these seven experiments, using in all 36 pots of buckwheat and 144 pots of alfalfa, did the differences in sulphur content of nutrient supply have a significant effect on susceptibility to injury by sulphur dioxide. No explanation can be offered for the significant interaction with water supply in experiment 4 a.

PRETREATMENT WITH SULPHUR DIOXIDE

Wells was the first to point out (8, p. 43) that, other conditions being equal, a given concentration of sulphur dioxide applied for a total period of time produces the same results whether the treatment is applied continuously or at short intervals, but that as the time interval between repeated applications is increased the injury produced becomes less. Thomas and Hill (21), going a step farther, have shown that leaf injury is approximately a linear function of rate of absorption.

Although no attempt has been made to set a time limit for complete recovery from an SO_2 treatment, it has been generally accepted that such a recovery does occur within a period of several days.

Recently, however, it has been suggested (22, p. 261) that any exposure of plants to sulphur dioxide brings about a greater susceptibility to subsequent SO_2 treatment even if the pretreatment was in a previous year. To test this point several experiments were performed on "accumulative and residual effects."

In experiment 2, 24 pots each of buckwheat and alfalfa were treated with an average concentration of 0.33 p.p.m. of SO_2 for 48 hours. After an interval of 96 hours with no SO_2, the treated buckwheat and 24 pots of untreated control plants were together treated with 0.95 p.p.m. of SO_2 for five hours. After an interval of 101 hours with no SO_2, the treated alfalfa and 24 pots of untreated control plants were together treated with 0.87 p.p.m. for five hours. Both five-hour runs caused considerable markings.

Two days after the conclusion of the five-hour treatments, the pots were rated according to degree of injury. The analyses of variance of the resultant data reveal no significant differences between the injury ratings of the plants pretreated with sulphur dioxide and the plants which had not been exposed to previous treatment.

In experiment 4 a, using in all 24 pots of alfalfa, pretreatment with sulphur dioxide consisted of one 118-hour fumigation at an average concentration of 0.19 p.p.m. The plants then were given a rest period of seven days before treatment with 0.91 p.p.m. of SO_2 for 5.5 hours. Half the plants were given no SO_2 pretreatment. There was no significant difference between the injury ratings of the pretreated and control plants.

In experiment 4 b, using in all 36 pots of alfalfa, the pretreatments with sulphur dioxide consisted of a 118-hour fumigation at an average concentration of 0.19 p.p.m. and of a 4-hour fumigation at an average concentration of 1.06 p.p.m. The plants pretreated with 0.19 p.p.m. were given a rest period of seven days before the final treatment with 0.91 p.p.m. of sulphur dioxide for 5.5 hours. The plants pretreated with 1.06 p.p.m. were given a rest period of 15 days before the final treatment.

The injury rating of the alfalfa pretreated with 1.06 p.p.m. for four hours was markedly less than was the injury rating of both the alfalfa which was not exposed to a sulphur dioxide pretreatment and the alfalfa which was pretreated with 0.19 p.p.m. for 118 hours. This possible explanation is offered: After pretreatment the "1.06 p.p.m. alfalfa" was placed on the greenhouse benches while the other alfalfa was in the SO_2 and control cabinets at higher humidities than the humidities which prevailed in the greenhouse. Such higher humidities were caused by the scrubbers attached to the blowers.

The resultant increased succulence of the cabinet plants may have been responsible for the increased sensitivity to sulphur dioxide. There was no significant difference between the injury rating of the non-pretreated plants and those pretreated with 0.19 p.p.m. for 118 hours.

Although these experiments prove that sulphur dioxide pretreatment does not necessarily predicate a greater susceptibility, more data are necessary before quantitative information on time for complete recovery can be given.

LIGHT INTENSITY

Investigators are in almost complete agreement that plants are more resistant to sulphur dioxide at night than in daylight, believing that the difference is due largely to differences in light intensity. Wells (25) reports that investigators of the American Smelting and Refining Company found that plants in complete darkness were from five to six times as resistant to sulphur dioxide as were plants in the light.

Wells (25), O'Gara (14), and Katz and Ledingham (12) further agree that light intensities above a certain minimum have no marked effect on susceptibility. Wells (25, p. 262) states " . . . the susceptibility of the grain (barley) to sulphur dioxide gas is not greatly affected by sunlight or shade." O'Gara found that plants began to show increased resistance whenever the light was as low as 2 per cent of maximum, but that there was no difference between 8 per cent and 60 per cent values. Katz and Ledingham conclude (12, p. 21) that "light in excess of about 3000 foot candles does not appear to have any marked influence on susceptibility. Below this value a drop in light intensity results in a decrease in the absorption of plants with a consequent increase in resistance to SO_2." O'Gara's figure of 8 per cent probably represents a light intensity of about 800 foot candles.

Janson (9), Wislicenus (28), and other German investigators assign great importance to light intensity but fail to recognize any critical intensity, believing that absorption of SO_2 is a direct function of light intensity in all ranges. These workers, however, base their conclusions solely on field observations and certain theoretical considerations, submitting no experimental data.

In the present series of experiments no attempt was made to treat plants with sulphur dioxide at various light intensities. Rather, several sets of plants were grown at different light intensities, then treated with sulphur dioxide in light of uniform intensity. No material has been found in the literature on experiments of this nature.

In experiment 4 c half of the buckwheat was grown from seed under atmospheric light conditions and half from seed in a cage of muslin. Two days before the sulphur dioxide fumigation, the shaded buckwheat was moved into frames with full light. The plants were 17 days old at time of SO_2 treatment. The plants grown with full light were not as tall, but were much more sturdy than the plants grown in the shade. The preshaded plants were the more susceptible, the difference in injury ratings being highly significant.

In experiment 5, one-third of the alfalfa was grown in a greenhouse whose glass transmitted the ultra-violet, and one-third under a double layer of cheesecloth in the same greenhouse, while one-third was grown in one of the cabinets in the SO_2 greenhouse, and consequently under two layers of window glass which transmitted the ultra-violet only feebly. The plants were exposed to these variations in light treatment for two weeks prior to fumigation with sulphur dioxide. By regulating the volume of air entering the cabinet, temperature and humidity were maintained nearly the same as in the greenhouse with Uviol glass.

There was no apparent difference in the appearance of the plants, and upon treatment with sulphur dioxide there resulted no significant differences in susceptibility.

In experiment 6, with buckwheat grown under the same conditions as the alfalfa in experiment 5, the shaded buckwheat injury ratings were significantly greater than those of the other two classes of buckwheat whose ratings were the same.

In experiment 11, 48 pots of buckwheat were grown from time of germination until time of sulphur dioxide treatment in frames heavily shaded with overlapping wooden slats; 48 pots were grown for the same period in frames shaded with wooden slats one inch wide, set one-half inch apart; and 48 pots were grown under normal atmospheric light conditions.

Thirty-six pots of each set selected at random were treated with sulphur dioxide. The injury ratings of the "heavy shade," "light shade," and "no shade" plants bore the relationship 79:66:87. The differences in injury ratings were just significant.

The remaining 12 of each 48-pot set were used to determine representative green and dry weights at the time of treatment with sulphur dioxide. The ratio of green weights of the leaves of the "heavy shade," "light shade", and "no shade" plants was 72:49:23. In an effort to get some quantitative measure of succulence, the ratios of air-dry weights to green weights were determined and found to bear the relationship 19:14:13.

In experiments 18, 22, and 28, the plants were grown in frames from time of germination until time of fumigation under one layer of heavy 44×40 unbleached muslin, under one layer of 34×32 cheesecloth, under one layer of 16×22 hospital gauze, or under no shading at all. Arthur and Stewart of this Institute report (1) that these three degrees of shading transmit 35 per cent, 65 per cent, and 75 per cent of the light, respectively.

The pots for fumigation in each of the experiments 18, 22, and 28 were selected at random from the same four sets of light-controlled plants. The ages of the plants at times of fumigation were 15, 21, and 25 days, respectively.

In each of the three experiments the effect of the various shade pretreatments on injury rating was highly significant. In each the muslin-shaded plants were much more susceptible than the other three sets which did not differ significantly in susceptibility. The effect of shading was less significant than the effects of water supply and of soil fertility in each of the three experiments.

It is interesting to note that only in the muslin-shaded area did the average daylight intensity fall below 3000 foot candles, the critical value set by Katz and Ledingham for light intensities at time of fumigation.

AGE OF PLANTS

Widstoe (26) reports that young plants and the young tender parts of plants are injured more than the older plants and plant parts. Wislicenus (28) and O'Gara (14) declare that young leaves are more resistant than older ones.

More recently, Hill and Thomas (7) state that almost invariably the fully-grown, highly functional leaves are more susceptible than the rest. Zimmerman and Crocker (30) found that middle-aged leaves of plants in an active state of growth were most susceptible. The young or growing leaves were found to be the most resistant. The observations of Katz and Ledingham (12, p. 16) support those of Hill and Thomas, and of Zimmerman and Crocker.

Wieler (27, p. 543) and Frazer (5), however, still maintain with Widstoe that young plants and their young tender parts are more seriously affected than the older and hardier plants or plant parts.

In the present series of experiments plants of several ages were grown under nearly similar conditions and as a combined group treated with sulphur dioxide. In experiment 6, mature alfalfa was much more susceptible than the young plants. The odds against the difference in injury ratings having occurred by chance were of the order of 10,000 to 1.

In experiments 20, 24, and 30 the plants were, respectively, 5, 11, and 19; 9, 15, and 23; and 11, 17, and 25 days old. In experiment 20 the youngest plants were only one to two inches tall, while the oldest plants averaged eight inches in height and were growing rapidly. In experiment 30, the youngest plants were about five inches tall, the oldest plants were about 20 inches in height and were just beginning to flower. In experiment 24, the oldest plants had not begun to flower. In each of these three experiments the oldest plants were most susceptible, the youngest plants the most resistant. The ratios of the injury ratings for the three experiments were, respectively, 42:90:131; 38:94:132; and 34:96:133. In each of the three experiments the differences in injury ratings due to age are highly significant and of greater magnitude than the differences due to any of the other variables. In experiments 20 and 24 the interaction age versus water supply during growth is highly significant because the differences in susceptibility due to water supply were greater for the younger plants in each case.

In experiment 29, buckwheat 38 days old which had been in flower for 10 days was much more susceptible than 22-day-old buckwheat which had not quite reached maturity. The interaction age versus soil fertility is highly significant because the differences in susceptibility due to soil fertility were greater for the older plants. The interaction age versus water supply is again highly significant because the differences in susceptibility due to water supply were greater for the younger plants.

MOIST LEAF SURFACES

It has often been claimed, by botanists in particular, that sulphur dioxide injury is associated with moist tissue surfaces—that the sulphur dioxide being highly soluble in water is dissolved out of the air and concentrated at the leaf surfaces. Swain (20) refutes this theory. "It is true," he declares (p. 298), "that this gas (sulphur dioxide) is very soluble in water—about 80 volumes of it dissolving in one volume of water, at standard conditions of temperature and pressure—but this solubility varies with the partial pressure of the gas in any gaseous mixture. In air containing 10 parts of sulphur dioxide per million parts of air, which is a high value for the smoke stream of a smelter at the level of vegetation, the partial pressure of the gas would be only one hundred thousandth of an atmosphere, and its solubility would be only one hundred thousandth of this amount. Not the slightest injury could possibly result from spraying plants with such dilute solutions of sulphur dioxide. In fact, at atmospheric temperature and at sea level, water through which air containing ten thousand parts of sulphur dioxide per million has been passed will not cause injury."

Nevertheless, to test the possibility of a surface reaction, four experiments were conducted to study the effect of moist surfaces on susceptibility. In each of three experiments, numbers 12, 16 a, and 16 b, the leaves on half of the plants were sprayed on the upper surface with distilled water several times in the course of the SO_2 treatment by means of a DeVilbiss No. 15 atomizer held about six inches above the leaf surfaces. The leaves were covered with as much moisture as possible without causing water to drip from the leaves, and the spray treatment was repeated as soon as the visible surface moisture had evaporated. In none of the experiments did the spray treatments cause a significant difference in susceptibility. The meaning of the significant interaction, moist surface versus water supply, in experiment 12 is not clear.

In experiment 28 both the upper and lower leaf surfaces were sprayed in a manner similar to that described above. The sprayed plants were more susceptible than the non-sprayed, but the difference in injury ratings was just barely significant. It would appear, therefore, that wetting the leaf surfaces has little or no effect on susceptibility.

OTHER VARIABLES

The factors of dark pretreatment, quality of light, time of day of sulphur dioxide treatment, and ethylene pretreatment, though included in certain of the experiments listed in Table IV, will be discussed in a subsequent paper together with other factors such as treatment with growth substances, exposure to formaldehyde and carbon dioxide, etc.

The various factors treated in the present paper might be termed environmental and those to be discussed in the subsequent paper might be termed physiological factors. The distinction is by no means rigidly drawn and there is of necessity some overlapping.

CONCLUSIONS AND SUMMARY

The influence of a number of environmental factors on susceptibility of certain plants to sulphur dioxide injury has been reviewed, and considerable new experimental data on susceptibility of alfalfa and buckwheat have been added to those phases of the problem which are treated less extensively in the literature. The new data have been obtained from 29 factorial experiments designed to study a number of variables simultaneously and have been subjected to statistical analysis by the method of the analysis of variance.

The influence of any environmental factor is based on observations of all the plants in each experiment. All the plants, furthermore, are utilized in ascertaining whether the effect of any environmental factor depends upon the absence or presence of other factors, that is, whether there exists any interaction between the factors. This method had been found to be a great improvement over the older methods of evaluating this type of data in which the tendency has been to direct attention to some particular case which happens to illustrate the case at hand. The experiments as designed have the further advantage that the effect of each factor rests on a broad basis of varied conditions rather than on some arbitrary standard running through the experiments.

The following statements summarizing the effects of the specific environmental factors, while based largely on the results of the factorial experiments, are founded in part on data previously reported in the literature:

1. A plant is much more resistant to sulphur dioxide at temperatures of 40° F. and below than at higher temperatures, yet there is no considerable variation in susceptibility above 40° F., at least not within the range of 65° F. to 105° F.

2. In general, resistance to sulphur dioxide decreases with increase in relative humidity though differences of at least 20 per cent appear necessary to cause detectable differences in susceptibility in the range above 40 per cent. No data are available on the effect on susceptibility of growing plants in various humidities prior to sulphur dioxide treatment.

3. Minor variations in soil moisture, at time of exposure to sulphur dioxide, the soil moisture being adequate for growth, are without effect on susceptibility, but when plants approach the wilting point there is a marked increase in resistance.

4. Plants grown with an ample supply of water are much more susceptible to injury than are plants grown with an inadequate supply of water, even if the soil moisture content is the same in both cases at time of fumigation.

5. Plants grown in a poor soil are more susceptible to injury than are plants grown in a good soil.

6. Sulphate sulphur content of nutrient supply has no effect on susceptibility.

7. Pretreatment with sulphur dioxide has no effect on susceptibility provided sufficient time is allowed for recovery between treatments.

8. Plants grown under heavy shade (65 per cent reduction in light intensity) are more susceptible to injury than are plants grown without shading. Reduction of light intensity up to 35 per cent has no effect on susceptibility.

9. Young plants are much more resistant to injury than are older plants. Middle-aged leaves are the most susceptible.

10. Wetting of leaf surfaces has little or no effect on susceptibility.

It is evident that many factors influence the susceptibility of plants to injury by sulphur dioxide, and that caution must be exercised in applying experimental results to conditions in the field. More quantitative experimental data on the effects of these various factors are necessary for their exact evaluation.

It is believed that the so-called physiological factors to be reported in the second paper in this series will shed more light on the mechanism of sulphur dioxide injury. It is further believed that eventually susceptibility to sulphur dioxide may be used as an indicator of certain physiological processes.

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SULPHUR DIOXIDE CONTENT OF AIR AT BOYCE THOMPSON INSTITUTE. II

Carl Setterstrom

In view of the importance of sulphur dioxide as an atmospheric contaminant, and because of widespread disagreement as to the harmful effects on plants of low concentrations of the gas, a continuous record of the sulphur dioxide concentrations prevailing in the atmosphere at Boyce Thompson Institute has been kept from November 1, 1936 to November 1, 1938, with minor interruptions.

Results of the recordings for the period November 1, 1936 to November 1, 1937 have been reported in a previous paper (3) which describes the methods used, sources of the sulphur dioxide, and condition of the vegetation. These phases of the problem will be mentioned only briefly in the present paper which will summarize the determinations for the 1937–38 period. Because of possible occasional contamination of air samples by nearby experimental fumigations with sulphur dioxide concentrations ranging from 10 to 110 parts per million parts of air by volume (p.p.m.), the recordings obtained during these fumigations, covering a total of 3694 hours, have been omitted from this report.

Automatic, continuous determination of sulphur dioxide concentrations was made possible by use of the sampling mechanisms or autometers, developed by Thomas and associates (4, 5, 6). Concentrations were continuously recorded on Leeds and Northrup Micromax conductivity recorders.

Correlation with wind direction indicates that the sulphur dioxide comes largely from New York City and environs (15.4 miles SSW to Times Square which marks the approximate center of the metropolitan area). Pincus and Stern (2) estimate that the amount of sulphur dioxide discharged into the atmosphere of New York City in 1934, exclusive of the discharge from internal combustion engines, averaged close to 2000 tons per day. Seasonal variation of concentration shows that heating plants are a source of much of the sulphur dioxide.

The many plants grown throughout the year in the Institute greenhouses and the vegetation in and about the Institute grounds are considered comparable with greenhouse plants and vegetation growing under similar conditions in areas free from sulphur dioxide.

TABLE I

Summary of Concentrations of SO_2 in the Atmosphere, at Boyce Thompson Institute, Yonkers, New York, November 1, 1937 to November 1, 1938

Month	Hours recorder was operating*	% time conc. was 0.01 p.p.m. and above	% time conc. was 0.10 p.p.m. and above	% time conc. was 0.20 p.p.m. and above	% time conc. was 0.30 p.p.m. and above	% time conc. was 0.40 p.p.m. and above	Max. conc., p.p.m.	Av. conc., p.p.m,
November	139	77.8	24.5	7.7	1.9	1.1	0.53	0.066
December	372	84.5	11.3	0.6	0.0	0.0	0.22	0.041
January	131	81.3	23.3	4.9	2.4	0.0	0.39	0.063
February	418	77.2	17.3	2.5	0.5	0.0	0.31	0.050
March	94	52.8	4.5	0.0	0.0	0.0	0.13	0.024
May	203	29.0	2.1	0.5	0.0	0.0	0.23	0.012
June	685	56.3	5.9	0.3	0.0	0.0	0.23	0.028
July	732	59.5	4.6	0.1	0.0	0.0	0.20	0.025
August	738	60.7	3.8	0.4	0.0	0.0	0.23	0.025
September	716	52.4	10.9	0.8	0.0	0.0	0.26	0.025
October	725	54.9	6.3	0.7	0.0	0.0	0.26	0.055
November 1, 1937 to November 1, 1938	4953	60.6	8.3	1.0	0.2	0.0	0.53	0.035

* Excluding periods of high concentration experimental fumigations. For list of omitted periods see footnote at end of Table II.

Results of the sulphur dioxide determinations are summarized in Table I. The more important occurrences of the gas are listed at considerable length in Table II. In neither of these tables have the determinations made during the high concentration experimental fumigations been included. For the times of the excluded periods see the footnote at end of Table II.

For the periods reported, the average concentration including zero readings was 0.035 p.p.m. Maximum concentration recorded was 0.53 p.p.m. Sulphur dioxide was present in concentrations of 0.01 p.p.m. and above, 60.6 per cent of the time. For the 1937–38 period the average concentration was 0.033 p.p.m., the maximum 0.75 p.p.m., and the gas was present 62.2 per cent of the time. Examination of meteorological reports (1) indicates that prevailing wind directions were much the same for both periods.

TABLE II

List of the More Important Occurrences of SO_2 in the Atmosphere at Boyce Thompson Institute

November 1, 1937 to November 1, 1938

Date	Time	Total hours of gas	Av. conc. in p.p.m.	Max. conc. in p.p.m.
Nov. 24 to 25	4:13 P.M.– 2:37 P.M.	22.4	0.062	0.13
Nov. 25 to 27	4:13 P.M.–11:30 A.M.	43.3	0.135	0.53
Nov. 29 to 30	1:47 P.M.–12:53 P.M.	23.1	0.061	0.25
Nov. 30 to Dec. 1	4:37 P.M.– 3:17 A.M.	10.7	0.040	0.08
Dec. 1 to 4	2:29 P.M.– 3:49 P.M.	73.3	0.059	0.16
Dec. 4 to 5	4:53 P.M.– 4:37 A.M.	11.7	0.064	0.16
Dec. 5 to 6	9:25 A.M.– 1:17 P.M.	27.9	0.081	0.20
Dec. 6	3:25 P.M.– 7:09 P.M.	3.7	0.040	0.10
Dec. 8 to 9	4:13 A.M.– 3:09 A.M.	22.9	0.066	0.22
Dec. 9 to 10	5:49 P.M.– 5:33 A.M.	11.7	0.044	0.13
Dec. 11 to 12	1:01 P.M.– 4:29 A.M.	15.5	0.027	0.06
Dec. 12	5:01 A.M.–10:05 P.M.	17.1	0.027	0.08
Dec. 12 to 14	10:37 P.M.– 4:29 A.M.	29.9	0.032	0.10
Dec. 14 to 15	6:05 A.M.–11:09 A.M.	29.1	0.040	0.09
Dec. 15 to 16	6:43 P.M.–12:51 P.M.	18.1	0.076	0.20
Jan. 27 to 28	3:49 P.M.– 9:09 P.M.	29.3	0.037	0.08
Jan. 28 to 29	10:13 P.M.–11:03 A.M.	12.8	0.052	0.08
Jan. 29 to Feb. 1	2:15 P.M.–12:39 P.M.	70.4	0.095	0.39
Feb. 2	12:33 A.M.– 1:42 P.M.	13.2	0.041	0.11
Feb. 3 to 4	3:50 P.M.– 6:14 A.M.	14.4	0.072	0.25
Feb. 4	6:46 A.M.–10:50 P.M.	16.1	0.030	0.10
Feb. 5 to 6	11:38 A.M.–11:54 P.M.	36.3	0.091	0.31
Feb. 8 to 10	2:58 P.M.– 3:30 A.M.	36.5	0.095	0.30
Feb. 10	6:10 A.M.– 5:25 P.M.	11.3	0.052	0.10
Feb. 11	8:52 A.M.– 2:12 P.M.	5.3	0.053	0.08
Feb. 12 to 13	8:04 P.M.– 2:12 P.M.	18.1	0.078	0.16
Feb. 13 to 14	5:24 P.M.– 1:38 P.M.	20.2	0.118	0.29
Feb. 14	4:18 P.M.–11:14 P.M.	6.9	0.043	0.10
Feb. 15 to 16	12:18 A.M.– 5:55 P.M.	41.6	0.054	0.12
Feb. 17 to 18	6:43 A.M.–10:59 A.M.	28.3	0.088	0.22
Mar. 16	7:45 A.M.–10:57 A.M.	3.2	0.050	0.08
Mar. 18	8:49 A.M.– 6:45 P.M.	9.9	0.083	0.13
Mar. 19	9:09 A.M.–11:49 A.M.	2.7	0.060	0.10
May 24	3:32 A.M.– 4:52 P.M.	13.3	0.072	0.23
May 24 to 25	11:16 P.M.– 4:04 A.M.	4.8	0.040	0.08
May 27	2:03 A.M.– 5:15 A.M.	3.2	0.062	0.10
May 31 to June 1	11:50 P.M.– 4:06 A.M.	4.3	0.071	0.20
June 1	11:02 A.M.– 3:50 P.M.	4.8	0.066	0.16
June 2	7:18 A.M.– 3:18 P.M.	8.0	0.077	0.18
June 3 to 4	6:01 P.M.–12:41 P.M.	18.7	0.049	0.10
June 5	10:17 P.M.–11:53 P.M.	1.6	0.087	0.22
June 6 to 7	7:31 P.M.– 9:09 P.M.	25.6	0.076	0.14
June 8	2:57 A.M.– 3:45 P.M.	12.8	0.048	0.11
June 9	2:25 A.M.– 9:53 A.M.	7.5	0.039	0.08
June 11 to 12	9:00 A.M.– 1:00 A.M.	16.0	0.047	0.11
June 12 to 13	9:52 P.M.– 2:24 P.M.	16.5	0.046	0.10
June 14	2:08 A.M.– 1:52 P.M.	11.7	0.054	0.11
June 15	4:16 A.M.–10:08 A.M.	5.9	0.059	0.13
June 18	8:28 A.M.– 6:36 P.M.	10.1	0.085	0.23
June 18 to 19	7:08 P.M.– 1:16 P.M.	18.1	0.048	0.14
June 20	3:08 A.M.– 1:26 P.M.	10.3	0.031	0.09
June 20 to 21	5:10 P.M.– 4:54 A.M.	11.7	0.044	0.14
June 21	6:32 A.M.– 4:42 P.M.	10.2	0.065	0.12
June 22	1:47 A.M.– 6:35 A.M.	4.8	0.032	0.09

TABLE II (*Continued*)

Date	Time	Total hours of gas	Av. conc. in p.p.m.	Max. conc. in p.p.m.
June 23	12:43 A.M.– 1:26 P.M.	12.7	0.074	0.13
June 23	4:06 P.M.–11:34 P.M.	7.5	0.036	0.12
June 24	3:55 A.M.– 4:11 P.M.	12.3	0.083	0.16
June 24 to 25	5:15 P.M.– 4:11 P.M.	22.9	0.060	0.20
June 25 to 26	5:47 P.M.– 3:55 A.M.	10.1	0.040	0.09
June 29 to 30	2:31 A.M.–10:07 A.M.	31.6	0.049	0.16
July 1	12:02 A.M.– 4:50 A.M.	4.8	0.041	0.09
July 1 to 2	12:50 P.M – 3:46 A.M.	14.9	0.030	0.05
July 8	3:26 A.M.– 3:42 P.M.	12.3	0.055	0.16
July 8 to 9	7:58 P.M.– 1:34 P.M.	17.6	0.053	0.11
July 11	4:16 P.M.– 9:04 P.M.	4.8	0.067	0.11
July 12	6:11 A.M.– 3:47 P.M.	9.6	0.035	0.09
July 14	4:07 A.M.– 4:55 P.M.	12.8	0.059	0.10
July 14 to 15	6:31 P.M.–11:03 A.M.	16.5	0.035	0.11
July 18 to 19	5:43 A.M.– 5:43 A.M.	24.0	0.053	0.13
July 19	7:33 A.M.– 4:05 P.M.	8.5	0.061	0.12
July 20 to 21	12:09 A.M.– 2:49 A.M.	26.7	0.055	0.20
July 22 to 23	10:56 A.M.– 1:52 A.M.	14.9	0.050	0.14
July 23 to 24	7:28 P.M.– 7:28 P.M.	24.0	0.048	0.12
July 25 to 27	10:50 P.M.– 1:57 P.M.	39.1	0.051	0.10
July 28	6:33 A.M.– 4:09 P.M.	9.6	0.065	0.11
July 29	1:18 A.M.– 5:50 P.M.	16.5	0.077	0.21
July 31	6:31 P.M.–10:15 P.M.	3.7	0.061	0.14
Aug. 1	12:59 A.M.– 7:07 P.M.	18.1	0.044	0.11
Aug. 3 to 4	7:45 P.M.– 2:24 P.M.	18.7	0.060	0.13
Aug. 4 to 5	8:48 P.M.– 2:29 P.M.	17.7	0.038	0.10
Aug. 6 to 7	3:17 A.M.– 4:53 A.M.	25.6	0.057	0.13
Aug. 7 to 8	6:13 P.M.–12:05 A.M.	5.9	0.046	0.09
Aug. 8 to 9	11:17 A.M.–12:05 A.M.	12.8	0.044	0.14
Aug. 9 to 10	8:40 P.M.–12:40 P.M.	16.0	0.036	0.06
Aug. 11	3:10 A.M.– 3:58 P.M.	12.8	0.037	0.08
Aug. 16	5:44 A.M.– 4:56 P.M.	11.2	0.045	0.09
Aug. 18	10:37 A.M.– 8:13 P.M.	9.6	0.038	0.08
Aug. 20 to 21	8:28 P.M.– 2:20 A.M.	5.9	0.053	0.13
Aug. 21	2:52 A.M.– 3:40 P.M.	12.8	0.041	0.10
Aug. 21 to 22	10:04 P.M.– 5:17 P.M.	19.2	0.048	0.20
Aug. 23 to 24	8:34 P.M.– 1:54 A.M.	5.3	0.056	0.11
Aug. 25	6:43 A.M.– 4:51 P.M.	10.1	0.051	0.09
Aug. 27	5:39 A.M.– 3:15 P.M.	9.6	0.052	0.10
Aug. 28 to 29	11:47 P.M.– 5:07 A.M.	5.3	0.072	0.15
Aug. 29 to 30	10:12 P.M.– 3:16 P.M.	17.1	0.065	0.13
Aug. 31	6:44 A.M.– 3:48 P.M.	9.1	0.111	0.23
Sept. 3	2:23 A.M.– 1:35 P.M.	11.2	0.078	0.16
Sept. 3 to 4	9:35 P.M.– 2:39 P.M.	17.1	0.048	0.17
Sept. 6 to 7	9:58 P.M.– 9:58 P.M.	24.0	0.086	0.24
Sept. 10	5:11 A.M.– 3:19 P.M.	10.1	0.046	0.10
Sept. 11	1:59 A.M.–11:03 A.M.	9.1	0.056	0.18
Sept. 12 to 13	2:46 A.M.– 5:10 P.M.	38.4	0.058	0.26
Sept. 15	5:03 A.M.–10:07 P.M.	17.1	0.065	0.14
Sept. 19	7:45 A.M.– 8:11 P.M.	12.4	0.030	0.07
Sept. 19 to 20	6:23 A.M.–11:00 P.M.	16.6	0.029	0.06
Sept. 21	4:52 A.M.– 3:39 P.M.	10.8	0.044	0.10
Sept. 24	1:11 A.M.–12:34 A.M.	11.4	0.075	0.12
Sept. 26	7:22 A.M.– 4:26 P.M.	9.1	0.060	0.16
Sept. 26 to 27	8:20 P.M.– 7:06 P.M.	22.8	0.055	0.12
Sept. 28 to 29	6:18 A.M.– 6:01 P.M.	35.7	0.056	0.16
Oct. 2	7:23 A.M.– 3:55 P.M.	8.5	0.053	0.11
Oct. 4	4:26 A.M.– 4:09 P.M.	11.7	0.070	0.12

TABLE II (*Continued*)

Date	Time	Total hours of gas	Av. conc. in p.p.m.	Max. conc. in p.p.m.
Oct. 5	3:22 A.M.–11:57 A.M.	8.6	0.049	0.08
Oct. 5 to 6	9:01 P.M.– 6:33 A.M.	9.5	0.049	0.10
Oct. 8	12:05 A.M.–12:21 P.M.	12.3	0.071	0.15
Oct. 11 to 12	8:15 A.M.– 5:46 P.M.	9.5	0.055	0.15
Oct. 12 to 13	3:26 A.M.– 2:21 P.M.	34.9	0.084	0.26
Oct. 15 to 16	11:21 P.M.–12:09 P.M.	12.8	0.059	0.14
Oct. 16 to 17	10:13 P.M.– 2:49 P.M.	16.6	0.053	0.15
Oct. 19 to 20	1:47 A.M.– 4:23 P.M.	38.6	0.067	0.26
Oct. 25 to 26	7:40 P.M.– 2:56 P.M.	19.3	0.049	0.14
Oct. 26 to 27	4:00 P.M.– 7:56 A.M.	15.9	0.088	0.30
Oct. 27	9:37 A.M.– 1:42 P.M.	4.1	0.071	0.14
Oct. 27 to 28	4:33 P.M.– 7:29 A.M.	14.9	0.059	0.14
Oct. 29 to 31	9:34 P.M.– 5:27 P.M.	43.9	0.069	0.15

Determinations for the following periods are not included in this report because of possible occasional contamination from nearby experimental fumigations at concentrations of 10 to 110 p.p.m.: 12:01 A.M. November 1, 1937 to 3:45 P.M. November 24, 1937; 1:10 P.M. December 16, 1937 to 1:10 P.M. January 26, 1938; 11:30 A.M. February 18, 1938 to 11:30 A.M. March 14, 1938; 11:30 A.M. March 19, 1938 to 8:19 A.M. May 23, 1938.

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A BIGENERIC GLADIOLUS HYBRID

FORMAN T. MCLEAN

In a series of cross pollinations between species and hybrid gladioli and gladiolus relatives, it was found that not all of the gladiolus species are interfertile, but that gladioli could be crossed with closely related genera in isolated instances. Bigeneric hybrids are sufficiently uncommon for most of them to have been recorded and discussed. Only one previous one with gladiolus has been reported to the author's knowledge. Luther Burbank is said to have crossed Watsonia with gladiolus, but no record of the results was published.

One bigeneric hybrid was flowered at the Boyce Thompson Institute last winter. It is a cross between *Antholyza revoluta* Burm., the Rood Kapje of South Africa, and the garden hybrid gladiolus "Byron L. Smith" (Kunderd), which was the pollen parent. The cross was made in 1935, in the late winter, using pollen of "Byron L. Smith" from a florist's flower shipped from Florida on pistils of *Antholyza revoluta* growing in the greenhouse at the New York Botanical Garden. The two parents are very different in appearance. "Byron L. Smith" is a large-flowered, pale lavender gladiolus of the ordinary type. *A. revoluta* strongly resembles the Afrikander type of species gladiolus, with cruciate leaves resembling *Gladiolus tristis* L., and a spotted basal sheath like *G. recurvus* L. Its flower has a long perianth tube, expanded in the middle part, and it is on this character that it is placed in the genus *Antholyza*. The segments of the perianth are rather narrow, pointed, spreading, and bright red, so that the flower is a six-pointed star, about one and one-half inches across, borne two or three on a slender, erect stalk, less than a foot tall. The whole plant so nearly resembled a red-flowered *G. tristis* that it seemed worthwhile to attempt crossing it with a gladiolus. It is a winter grower and early bloomer, both in its natural home and in our greenhouses.

The set of seed from the pollinations was ample, and a large number of seedlings are now growing. For the most part, they have broad leaves and rather strong growth, more resembling the gladiolus pollen parent. The three that have flowered, two of which are shown in the accompanying illustration (Fig. 1), also have flowers like the garden gladiolus, both in form and colors. One is a pale flesh-pink, the second a salmon-pink, and a third was dark maroon red. The first and largest bloom was four inches across, the equal of the gladiolus parent in both size and shapeliness.

The influence of the seed parent is shown in the winter-growing habit of the seedlings, all of which have regularly made their growth in winter and died down during the summer months. Even plants of these hybrids started into new growth in spring have died back in summer, only to renew active growth in the cool weather of autumn. Also, the influence of the wide outcross is further shown in the complete sterility of the three hybrids flowered. All have failed to set seed with pollen of either garden gladioli or with Afrikander gladiolus hybrids. They also appear to have no viable pollen of their own.

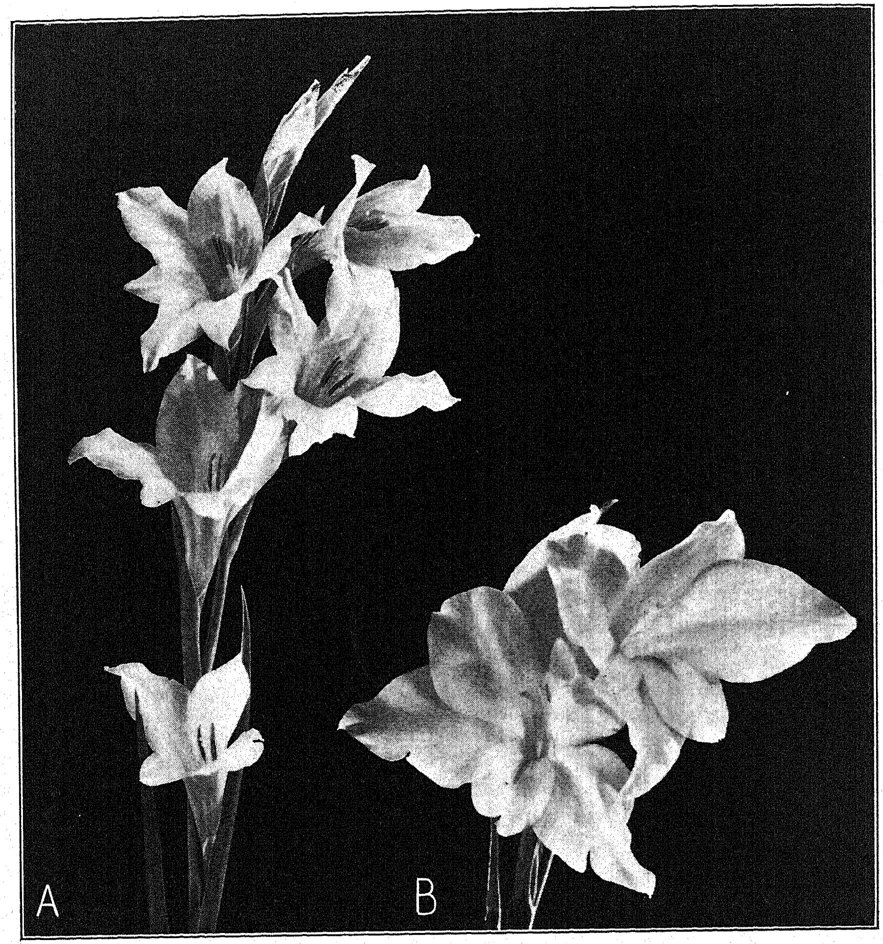

FIGURE 1. *Antholyza revoluta*×gladiolus "Byron L. Smith." A. This hybrid has salmon-pink flowers about three inches across. B. The flower is about as large as the gladiolus parent, and pale flesh-pink.

Because of their winter-blooming habit, some of these hybrids may have merit for commercial production of winter bloom in greenhouses or outdoors in the South.

LEAF-EPINASTY TESTS WITH CHEMICAL VAPORS[1]

F. E. DENNY

Since the production of leaf-epinasty is often used as an indication of the presence of ethylene in the air surrounding the leaf at the time the epinasty occurs, it is desirable to find the behavior of the leaf with respect to various volatile substances in order to learn whether this response is induced by only ethylene and perhaps a few other gases, or whether the reaction may be brought about by many volatile substances.

Crocker, Zimmerman, and Hitchcock (1) tested 38 gases and found only five of these to be effective: ethylene, acetylene, propylene, carbon monoxide, and butylene. Other chemicals not tested by these investigators but reported as inducing leaf-epinasty are formamide and acetonitrile in experiments by Wächter (5), and amylene, mesityl oxide, and vinylacetate in experiments by Nelson and Harvey (4).

In the present tests 87 volatile chemicals, including only the above-named five of those reported upon by Crocker, Zimmerman, and Hitchcock (1), and including the five listed by Wächter (5) and by Nelson and Harvey (4), were tested for epinasty.

METHODS

A measured amount of the chemical was placed in a small watch-glass which was enclosed with the potato test-plant in glass containers such as desiccators or bell-jars. A preliminary tests was made with each chemical to determine the concentration which would cause injury to the potato plant, and in subsequent experiments the amount of chemical was progressively reduced in series, the steps of which were usually one-half or one-third. The time allowed for the development of epinasty was 24 to 48 hours.

The test plants were young potato (*Solanum tuberosum* L.) plants grown in flats in the greenhouse until about three inches high and having at least one pair of well expanded leaves. The potato plants were cut off at the surface of the soil and placed in small vials with 5 cc. of H_2O, being held in place by pieces of cotton.

VOLATILE CHEMICALS NOT INDUCING EPINASTY

Substances not inducing epinasty were as follows: In the aliphatic series, hydrocarbons: amylene (C_5H_{10}), trimethylethylene $(CH_3)_2CCHCH_3$; halogen derivatives of hydrocarbons: methylene chloride CH_2Cl_2, methylene bromide CH_2Br_2, ethylene bromide CH_2BrCH_2Br, propylene bromide

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[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 185.

$CH_3CHBrCH_2Br$, ethylene chlorobromide CH_2ClCH_2Br, butylene bromide $C_2H_5CHBrCH_2Br$, amylene chloride $C_5H_{10}Cl_2$, dichloroethylene $CHClCHCl$, dibromoethylene $CHBrCHBr$, trichloroethylene CCl_2CHCl, tribromoethylene CBr_2CHBr, trichloroethane $CH_2ClCHCl_2$, tribromoethane $CH_2BrCHBr_2$, trimethylene bromide $(CH_2)_3Br_2$, allyl chloride CH_2CHCH_2Cl, allyl bromide CH_2CHCH_2Br, chloroform $CHCl_3$, carbon tetrachloride CCl_4; alcohols: n-butyl alcohol $C_2H_5CH_2CH_2OH$, β-γ-dibrom propyl alcohol $CH_2BrCHBrCH_2OH$, amyl alcohol $C_5H_{11}OH$, capryl alcohol $CH_3(CH_2)_5CHOH\ CH_3$, geraniol $C_{10}H_{18}O$; ethers: diisopropyl ether $C_6H_{14}O$, n-amyl ether $(C_5H_{11})_2O$, epichlorhydrin $CH_2Cl\ CHCH_2O$, chloroacetal $ClCH_2CH(OC_2H_5)_2$, methylal $CH_2(OCH_3)_2$, ethylal $CH_2(OCH_2CH_3)_2$, dichloroethyl ether $C_4H_8Cl_2O$; aldehydes: aldol $CH_3CHOHCH_2CHO$, crotonaldehyde $CH_3CHCHCHO$; ketones and derivatives: mesityl oxide $(CH_3)_2C{:}CH \cdot CO \cdot CH_3$, acetone $CH_3 \cdot CO \cdot CH_3$, chloracetone $CH_3 \cdot CO \cdot CH_2 \cdot Cl$; esters: iso-amyl formate $HCOO \cdot C_5H_{11}$, iso-amyl propionate $C_2H_5COOC_5H_{11}$, ethyl nitrate $C_2H_5NO_3$, vinyl acetate $CH_3COOCH{:}CH_2$, amyl acetate $CH_3 \cdot COOC_5H_{11}$; glycols and derivatives: ethylene glycol $CH_2OH \cdot CH_2OH$, trimethylene chlorhydrin $CH_2Cl \cdot CH_2 \cdot CH_2OH$, trimethylene bromhydrin $CH_2Br \cdot CH_2 \cdot CH_2OH$, propylene chlorhydrin $CH_3CHOHCH_2Cl$, propylene bromhydrin $CH_3CHOHCH_2Br$; cyanogen and thiocyanogen compounds: β-hydroxypropionitrile $HO \cdot CH_2 \cdot CH_2 \cdot CN$, methyl thiocyanate CH_3SCN, ethyl thiocyanate, C_2H_5SCN; other aliphatic compounds: nitromethane $CH_3 \cdot NO_2$, methyl sulphide $(CH_3)_2S$, formamide $HCO \cdot NH_2$; (and in addition to these, the chemicals in Table I giving negative tests for epinasty).

In the carbocyclic series: benzene derivatives: salicylaldehyde $HOC_6H_4 \cdot CHO$, fluorobenzene C_6H_5F, isopropyl benzene $C_6H_5CH(CH_3)_2$, o-chlorophenol ClC_6H_4OH, mesitylene $C_6H_3(CH_3)_3$, phenol C_6H_5OH, benzyl chloride $C_6H_5 \cdot CH_2Cl$, chlorobenzene C_6H_5Cl, o-cresyl methyl ether $CH_3C_6H_4OCH_3$, acetophenone $C_6H_5 \cdot CO \cdot CH_3$; hydroaromatic derivatives: phellandrene $C_{10}H_{16}$, cyclohexanol $C_6H_{11}OH$; cyclohexene C_6H_8, cyclohexanone $C_6H_{10}O$; naphthalene derivative: chloronaphthalene $C_{10}H_7Cl$.

In the heterocyclic series: furfural C_4H_3OCHO, and furfuryl alcohol $C_4H_3OCH_2OH$.

VOLATILE CHEMICALS INDUCING EPINASTY

Special care was taken in testing formamide, acetonitrile, amylene, mesityl oxide, and vinyl acetate, since these had been reported previously to have induced epinasty. Many trials of each chemical over a wide range of concentrations were made but only with acetonitrile (CH_3CN) were positive tests for epinasty of potato leaves obtained. A suitable concentration of acetonitrile was three drops in a 16-liter bell-jar.

Three other volatile chemicals caused epinasty: ethyl bromide (C_2H_5Br), using 10 drops in a 7-liter bell-jar; ethyl iodide (C_2H_5I), using 3 drops in a 16-liter bell-jar; and propyl chloride (C_3H_7Cl), using one cc. in a 3.5-liter desiccator. After the effect with ethyl bromide was noted, a test was made of various members of the alkyl halide series, the normal form of the propyl, butyl, and amyl representatives being chosen. The results given in Table I show that the property of inducing epinasty is not a group characteristic, only the three members named above showing consistently positive tests.

TABLE I

PRODUCTION OF EPINASTY BY ALKYL HALIDES

Alkyl \ Halide	Chloride	Bromide	Iodide
Methyl	−	−	−
Ethyl	−	+	+
Propyl	+	−	−
Butyl	−	−	−
Amyl	−	−	−

Note: + means that epinasty of potato leaves was obtained; − means that epinasty was not obtained.

The question arose whether the epinasty obtained with these four chemicals (acetonitrile, ethyl bromide, ethyl iodide, and propyl chloride) was due to traces of ethylene dissolved in the chemicals.

To test this point, a mixture of air and the vapors of each of these chemicals was passed slowly through a glass tube immersed in a Dewar flask containing carbon dioxide-snow and alcohol. If the epinasty was due to ethylene occurring in the chemical as an impurity, epinasty should be obtained with the uncondensed portion of the gas, since ethylene is not condensed at the temperature of carbon dioxide-snow.

Mixtures of air and the chemical vapors of these four chemicals were prepared in two-liter filter flasks, using enough of the chemical to give at least twice the concentration needed to produce epinasty. By water displacement, using a dropping funnel, this mixture was pushed slowly through the tube immersed in the freezing mixture, the emerging gas being led to the bottom of a 700 cc. desiccator containing two potato test plants in small vials of water. Epinasty was not obtained by this uncondensed gas in the case of any of these four chemicals. The water-solubility of acetonitrile is so great that it cannot be displaced from a flask in the manner described above, but ethylene even in a concentration of one part in 20 million by volume can be transferred without losing enough of the gas to prevent the production of epinasty. Failure to obtain epinasty after passage through the mixture is evidence that the observed result with the vapors of these four chemicals is not due to the presence of ethylene as a contamination.

It seemed possible that even if ethylene had been present as an impurity, it might redissolve in the condensed liquid within the tube in the freezing mixture, and thus be removed from the gas stream, even though ethylene itself does not condense at that temperature. To test this point a mixture of ethyl bromide (in an amount sufficient to give eight times the concentration needed to cause epinasty) and ethylene (in an amount to give one part in 20 million by volume) was prepared in a two-liter filter flask, and this mixture was pushed through the tube in the CO_2-snow-alcohol mixture in the Dewar flask. Epinasty was obtained with the air which had passed through the freezing mixture, which shows that ethylene was not removed from the air by the condensation of the vapor of ethyl bromide as it passed into the freezing mixture tube. This substantiates the conclusion that the epinasty obtained with acetonitrile, ethyl bromide, ethyl iodide, and propyl chloride is due to the vapors of these chemicals and not to ethylene present as an impurity.

CHARACTERISTICS OF EFFECTIVE CONSTITUENT FROM PLANT TISSUE COMPARED WITH VARIOUS VOLATILE CHEMICALS

Using the freezing mixture apparatus described in the preceding paragraph, tests were made of the air surrounding zinnia (*Zinnia elegans* Jacq.) seedlings, using 5 g. of seeds in a 2-liter flask, and closing the flask for two days after the seedlings had reached a height of one inch. After the air had passed through the freezing mixture and had been freed of its CO_2 content (due to respiration of the tissue) by absorption with alkali, it retained its capacity to induce epinasty of potato leaves. A similar result was obtained with lettuce (*Lactuca sativa* L.) leaves using 10 g. of tissue in a 2-liter flask for 24 hours, and with petals of petunia (*Petunia hybrida* Vilm.) using 5 g. of tissue. The behavior of the constituent from plant tissue is, therefore, quite different from that of acetonitrile, ethyl bromide, ethyl iodide, and propyl chloride, and this indicates that, although these four chemicals can induce epinasty, none of them is the effective constituent produced by plant tissue.

We have yet to consider, however, the possibility that one of four other volatile chemicals (propylene, acetylene, butylene, or carbon monoxide) may be produced by plant tissue and may be responsible for the epinasty. Evidence on this point was obtained by the use of the mercuric nitrate reagent used by Hansen and Hartman (3), and in a modified form in a previous experiment by the present writer (2). Ethylene is absorbed by this reagent and is released as a gas when HCl is added. A previous report (2) showed that the constituent of the air surrounding the seedlings of various species, gave a positive test for epinasty after its absorption by the mercury reagent and its releasal from it.

It seemed desirable to test the response of these chemicals to this reagent under conditions similar to those that obtain in these tests, that is, at low concentrations. Using propylene at concentrations of one part in 20,000, butylene at one part in 1,000, acetylene at one part in 20,000, carbon monoxide at one part in 1,000, ethylene at one part in 1,000,000, it was found that only with ethylene was epinasty obtained after absorption in the $Hg(NO_3)_2$-HNO_3 reagent and release by HCl.

SUMMARY

Of 77 volatile chemicals not previously tested for ability to induce epinasty of potato leaves, only three gave positive responses: ethyl bromide, ethyl iodide, and propyl chloride.

The epinasty-inducing volatile product from various organs of plants cannot be any of the three above-named alkyl halides, nor acetonitrile (previously reported to induce epinasty) since mixtures of these with air, when passed through a tube immersed in a freezing mixture of carbon dioxide-snow and alcohol, gave negative tests for epinasty, while the volatile products from plants were not condensed by the freezing mixture and the uncondensed gas which issues from the tube retained its effectiveness.

Tests of the other volatile chemicals which have been shown to cause epinasty, that is, ethylene, propylene, acetylene, butylene, and carbon monoxide, showed that the only one which behaves like the effective volatile constituent from plant tissue in being absorbed by the mercuric nitrate reagent and released again without loss of epinasty-inducing power was ethylene.

These results indicate that the effective constituent from plant tissue is ethylene and not any of the other epinasty-inducing volatile chemicals tested in these experiments.

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ACTIVATION OF CINNAMIC ACID BY ULTRA-VIOLET LIGHT AND THE PHYSIOLOGICAL ACTIVITY OF ITS EMANATIONS

P. W. Zimmerman and A. E. Hitchcock

Cinnamic acid (phenylacrylic acid) is known to exist in *cis* and *trans* isomeric forms. With ultra-violet irradiation *trans* cinnamic acid is converted into *cis* cinnamic, truxillic, and truxinic acids, the amounts of each varying with the solvents, temperature, and wave lengths of the light (Gilman 2, v. 1, p. 379). Cinnamic acid was first reported as an active growth substance by Hitchcock in 1935 (4) using a variety of test objects. Haagen Smit and Went (3) reported allo cinnamic acid active on *Avena* and *Pisum* but found no activity from *trans* cinnamic acid.

The sensitivity of cinnamic acid to light makes this compound an interesting subject for physiological investigations. Recent experiments have uncovered some unusual facts when plants were exposed to the action of cinnamic acid under varying conditions. This paper reports the response of plants to the different forms of cinnamic acid applied as vapors, as water solutions, and as lanolin preparations.

The commercial product known as cinnamic acid is a relatively inactive form as shown by its failure to induce pronounced epinasty of leaves of plants which are sensitive to growth substances. If, however, the chemical is mixed with lanolin and applied to the stems or leaves of plants in sunlight, curvatures and epinasty soon occur. Similar treatments of plants in the dark did not induce these responses. Since the plants in light showed a delayed response the assumption is that light activated the chemical by making the *cis* form which is physiologically active.

The effective wave lengths are in the region of the ultra-violet. Proof of this fact was obtained by treating plants in five spectral greenhouses, the glass of which varied in their transmission of ultra-violet light. The greatest response resulted in the house covered with Corex B glass which is known to transmit most of the ultra-violet light. No response occurred under red glass (Noviol C) transmitting to 472 mμ known to prevent the passage of violet and ultra-violet light.

Further evidence that the activation phenomenon was of a photochemical nature was obtained by irradiating the chemical before it was applied to the plants. For this purpose *trans* cinnamic acid was dissolved in an alcoholic water solution and irradiated with an ultra-violet lamp while covered with cellophane and three different kinds of glass. After irradiation the chemicals, in lanolin preparations, were applied to the plants in the dark. As in the spectral glass houses, the greatest activation occurred under covers transmitting the most ultra-violet light. Cellophane and a blue-purple glass (Corex A) were the most effective. The chemical solutions, irradiated while in flat dishes covered with cellophane for two hours and then uncovered and irradiated until dry, were very satisfactory, and this method was finally adopted as the standard.

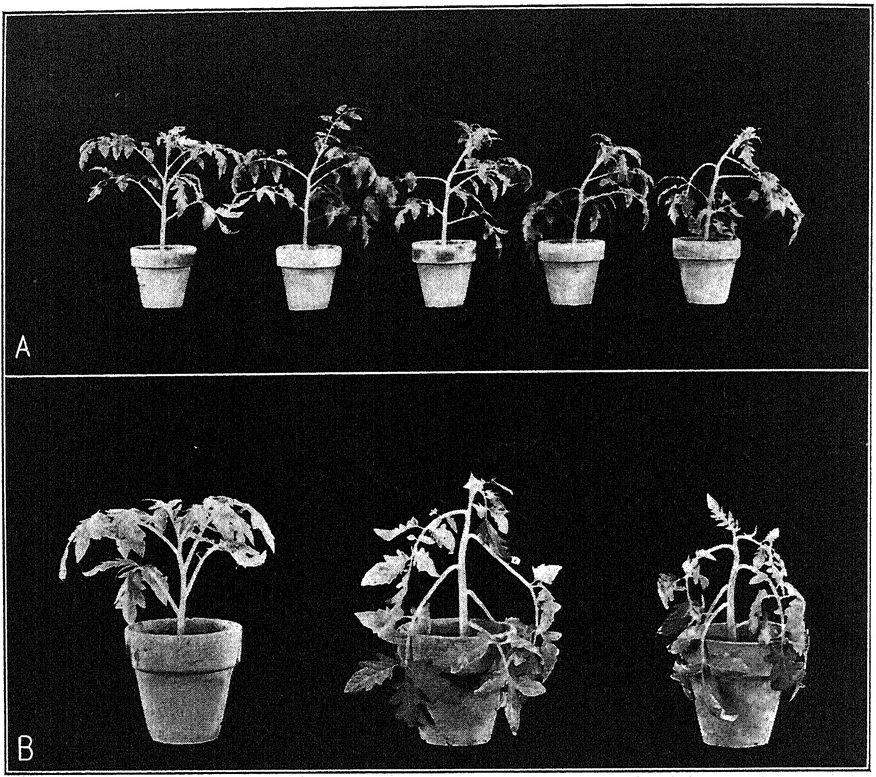

FIGURE 1. Tomato plants showing responses to irradiated (ultra-violet) cinnamic acid and ethylene gas. A. Plants treated with lanolin preparations containing the irradiated (but not extracted) cinnamic acid. Left to right, control; 0.2 per cent lanolin preparation applied on upper side of one petiole (left) and on one side of stem above the petiole; 0.4 per cent preparation; 0.6 per cent preparation; 1.0 per cent preparation. B. Plants treated under bell jars with ethylene gas and vapors arising from irradiated cinnamic acid crystals. Left, control; middle, approximately 1 to 1,000,000 ethylene gas; right, approximately 500 mg. of crystals in open petri dish under bell jar but not in direct contact with the plant.

Commercial cinnamic acid is a white crystalline substance. After the solution was irradiated and evaporated to dryness, crystals had a greenish tinge. When mixed with lanolin this form of cinnamic acid induced epinasty of tomato (*Lycopersicon esculentum* Mill.) leaves when in low concentrations (Fig. 1 A), as often reported for the most effective growth substances (5, 6). Also when these crystals were dissolved in water and the solution injected into the stem, the leaves showed epinasty, the degree of response varying with the concentration of the chemical. Leafy tomato cuttings set with the basal ends in the solution showed epinasty of all the leaves in the course of three hours. These cuttings later showed induced rooting as described for the other effective growth substances.

An active fraction was obtained by extracting the irradiated crystals with cold water and then evaporating the solution to dryness. This fraction when tested quantitatively as lanolin preparations was effective when the concentration was as low as 0.01 per cent. The water extract was approximately ten times as effective as the original mixture after irradiation.

The most surprising results were obtained by placing plants under a bell jar with irradiated cinnamic acid. Emanations from the crystals induced epinasty of leaves (Fig. 1 B) very much like the unsaturated hydrocarbon gases (1). The plants in the bell jar with the acid showed slightly more curling of the petioles and bending of the stems but these differences varied with the length of exposure. An exposure for one hour of a six-inch tomato plant in a 12-liter bell jar with 500 mg. of crystals spread out in a small petri dish, was sufficient to induce a pronounced response after the plant was removed. No effort has been made to determine the minimum dosages which will induce evident responses. There was no apparent loss in volume of crystals and the same set was used several times, indicating that very small amounts of the active substance escaped into the air.

By sublimation at different temperatures irradiated cinnamic acid produced several different fractions. The most active fraction was collected in partial vacuum at a temperature of 95° C. This is the temperature at which *cis* cinnamic acid is known to sublimate. Three *cis* forms with melting points of 42°, 58°, and 68° have been determined (2). The *trans* form has a melting point of 133° C. In our tests, the bulk of the sublimated fraction melted at 70° C.

This is the first crystalline growth substance found to have a sufficient vapor pressure to be effective as a gas for inducing responses similar to those caused by ethylene gas. Since some species of plants are known to produce cinnamic acid and store it in considerable quantities, the question arises as to whether this compound might play a special rôle as a natural growth substance.

Three other phenyl compounds, *p*-, *m*-, and *o*-nitrocinnamic acids, were activated by ultra-violet irradiation, but the resulting substances were not as active as that of cinnamic acid.

Beta-naphthoylacetonitrile and tryptophane became active after ultraviolet light treatment.

Phenylacetic acid which was known to be an active substance when applied in water or lanolin was found to be slightly active as a vapor.

SUMMARY

Commercial cinnamic acid is a relatively inactive compound for inducing curvatures but it can be activated by ultra-violet light. The *cis* form results from irradiation, and is the active form. When applied to plants as water solution or lanolin preparations this compound induces responses similar to other growth substances reported in earlier publications. In addition, the irradiated cinnamic acid induces epinastic responses of plants similar to ethylene gas when the crystals are set under a bell jar with the plants.

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CARBON DIOXIDE STORAGE. XIII. RELATIONSHIP OF OXYGEN TO CARBON DIOXIDE IN BREAKING DORMANCY OF POTATO TUBERS

Norwood C. Thornton

In a previous report (3) it was shown that carbon dioxide was effective in breaking the dormancy of freshly-harvested potatoes. Although the carbon dioxide treatments were carried out in the presence of oxygen, this has been considered by Quetel (1, p. 122) as an anaerobiosis treatment. Since the previous report, this investigation has been continued because at that time it was observed and stated in the report (3, p. 477) that the carbon dioxide treatment was more effective in the presence of 20 per cent than in 10, 5, or 0 per cent of oxygen. This observation has been repeated with the additional fact that the carbon dioxide is even more effective if higher percentages of oxygen are employed thus proving that the carbon dioxide treatment for breaking the dormancy of potatoes cannot be classed as one of anaerobiosis.

MATERIAL AND METHODS

Freshly-harvested potatoes (*Solanum tuberosum* L.) of the varieties Bliss Triumph and Irish Cobbler were obtained from the Institute garden and placed under treatment immediately and after holding at 25° C. for one week. The tubers were treated whole, both with and without removing the skins. The tubers were held in both a moist condition (in moist sphagnum moss) and a dry condition (without moss) during the 5 to 8 day period of the treatment at 25° C. The tubers, 8 to 16 in number, were placed in 8- or 18-liter tin containers having tight-fitting lids that were sealed with a mixture of paraffin and vaseline. The equipment and method of treating the potatoes with the gas mixtures composed of various percentages of carbon dioxide (0, 10, 20, 30, 40, 60, 80, and 100%), oxygen (0, 10, 20, 40, 80, and 100%), and nitrogen, as well as the gas analyses of the storage atmosphere, was the same as described in previous reports (2, 3).

The gas mixtures were renewed every 24 hours and the mixtures were frequently checked by gas analyses to insure that the potatoes were exposed to the desired percentages of oxygen and carbon dioxide.

At the end of the 5 or 7 day period of treatment the tubers were planted one and one-half to two inches deep in soil in order to observe the rate of emergence of sprouts. In some of the tests the tubers were planted whole while in most cases the tubers were cut into one-eye pieces previous to planting. Data were taken on the rapidity of sprout emergence, the time necessary for 50 per cent of the pieces to show sprouts above the soil, and for maximum sprouting to be obtained. These experiments were conducted over a period of three years with both varieties of potatoes.

RESULTS AND DISCUSSION

As shown in Table I, the most effective gas mixtures for breaking the dormancy of the potato tubers consisted of 10 to 60 per cent of carbon dioxide with 20 to 80 per cent of oxygen. Not only did germination occur first with one-eye pieces from tubers treated with these mixtures (Exp. 1 to 6 inclusive), but also 50 per cent emergence of sprouts was first obtained as a result of these treatments. These results were obtained irrespective of the variety of potato.

TABLE I

PERCENTAGES OF CARBON DIOXIDE AND OXYGEN FOUND MOST EFFECTIVE IN BREAKING THE DORMANCY OF FRESHLY-HARVESTED POTATOES, AND THE RATE OF SPROUT EMERGENCE AFTER PLANTING IN THE SOIL

Experiment number			1	2	3	4	5	6	7	8
Variety of potato*			IC	IC	BT	IC	BT	IC	BT	IC
Days in treatment			7	5	7	7	5	5	7	8
Best treatment	Percentages of	O_2 CO_2	40 10	20 40	80 20	80 20	20 30	20 60	20 60	40 40
	Germination, days after harvest**	First sprout 50% emergence	16 17	16 28	25 28	25 28	21 28	24 30	30 60	25 60
Second best treatment	Percentages of	O_2 CO_2	20 20	40 20	20 30	20 30	10 60	40 30	80 10	20 60
	Germination, days after harvest**	First sprout 50% emergence	16 19	16 30	25 34	25 34	21 38	24 33	37 60	25 70
Third best treatment	Percentages of	O_2 CO_2	20 20	10 80	10 30	10 30	10 30	80 20	10 60	10 60
	Germination, days after harvest**	First sprout 50% emergence	16 19	16 35	27 40	27 45	24 40	24 43	42 60	55 70
Nitrogen treatment	Germination, days after harvest**	First sprout 50% emergence	R† R†	18 61+	27 40	27 54	30 44	26 66	35 60+	60 78
Control	Germination, days after harvest	First sprout 50% emergence	38 53	61 61+	37 50	49 78	30 44	38 75	38 60	60 78

* IC = Irish Cobbler; BT = Bliss Triumph.
** Includes the period of treatment.
† R = Rotted in treatment.

The second best germination results were obtained in seven out of eight tests from potatoes that were likewise treated with carbon dioxide and normal (20 per cent) or higher percentages of oxygen. The second best treatments were considerably different from the first in respect to the amount of carbon dioxide and oxygen necessary to bring about the sprouting of the dormant potatoes, thus showing that the treatments over a wide range of gas concentrations were almost equally effective. As shown by the data in Table I, 50 per cent of the treatments effective in producing both first and second best germination consisted of 20 per cent of oxygen with various percentages of carbon dioxide and in only one case, second best germination in Exp. 5, did a treatment with 10 per cent of oxygen with carbon dioxide produce early germination of the potatoes. And not even this concentration of oxygen approaches a condition of anaerobiosis.

The less effective concentrations of carbon dioxide and oxygen brought about the breaking of dormancy of the potato tubers with a slow emergence of sprouts slightly in advance of the tubers that had received no treatment. The untreated tubers required 50 days or more to produce 50 per cent emergence of sprouts.

Tubers held in the presence of nitrogen without added oxygen during the period of treatment produced sprouts at about the same rate as the third best treatment with carbon dioxide and slightly in advance of the untreated tubers as shown in Table I.

The effectiveness of the carbon dioxide-oxygen mixtures in breaking the dormancy of freshly-harvested potatoes was evident regardless of the conditions of the treatment, whether the whole tubers were held with or without removing the skins in either a moist or a dry condition. Under those conditions that normally promote early sprouting (skins removed or moist storage) the carbon dioxide-oxygen treatments also brought about earlier sprouting than was obtained with the tubers held in a normal amount of oxygen (20 per cent) without carbon dioxide.

The tubers planted whole after treatment with carbon dioxide and normal or high percentages of oxygen were those in Exp. 7 and 8, Table I, which also showed sprouting in advance of the tubers held as control. No doubt the treatment of the whole tubers should have been conducted for a longer period of time before planting in the soil since it is usually more difficult to obtain sprouting of whole tubers than one-eye pieces of tubers.

The data from all the experiments conducted during these tests show a definite trend in the results; a higher concentration of carbon dioxide was necessary to break the dormancy of potatoes in a low than in a high concentration of oxygen. The optimum concentrations of carbon dioxide found effective in the presence of various percentages of oxygen in causing a shortening of the rest period of the potato (10% O_2 and 60 to 80% CO_2, 20% O_2 and 20 to 60% CO_2, 40% O_2 and 10 to 40% CO_2, and 80% O_2 and 10 to 20% CO_2) indicated some relationship with the rate of respiration of the potato tissue. As previously observed (2, 3), carbon dioxide increases the respiration and general metabolism of the potato tuber during treatment and the carbon dioxide is more effective in increasing the rate of respiration of the tubers in a high per cent than in a low per cent of oxygen.

From these results one cannot conclude that carbon dioxide breaks the dormancy of freshly-harvested potatoes as a result of a condition of anaerobiosis because in every treatment and especially in the more effective treatments there was always present in the atmosphere of the storage container more than sufficient oxygen to prevent the development of an anaerobic condition. One must not consider carbon dioxide as an inert or neutral gas but as an active regulator of the metabolism of living tissue.

SUMMARY

The elimination of the dormancy of freshly-harvested potatoes by treatment with carbon dioxide is not due to the development of a condition of temporary anaerobiosis during treatment as has been suggested by Quetel. Instead, carbon dioxide is most effective in breaking the dormancy of potato tubers when it acts in the presence of 20 per cent or more of oxygen and this treatment is more effective than a completely anaerobic condition brought about by treatment with nitrogen. The optimum concentrations of carbon dioxide are 10 to 60 per cent in combination with 20 to 80 per cent of oxygen which will, with a period of 5 to 7 days of treatment, cause the emergence of sprouts from 50 per cent of one-eye pieces in soil within 17 to 30 days as compared with 44 to 78 days for the controls.

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A FURTHER REPORT ON THE STORAGE OF VEGETABLE SEEDS

Lela V. Barton

INTRODUCTION

The first report (2) on the storage of these vegetable seeds contained a review of the pertinent literature. Some additional publications dealing with the same subject have appeared since 1935.

Rodrigo (8) found that thoroughly air-dried farm crop seeds kept better in sealed than in open containers at room temperature. Some vegetable seeds were stored by San Pedro (9) with and without calcium chloride at temperatures from 0° to 28° C. The beneficial effect of drying was significantly shown by all seeds after 312 days in storage with the exception of tomato seeds which kept well in all conditions. From his results the author concludes that dry, warm storage was better than moist, cool storage. Hence he considered moisture more detrimental than temperature.

The effect of moisture and temperature on the keeping quality of onion seeds has been studied by Beattie and Boswell, who reported (4) that at 40° F. and room temperature storage, 10 per cent moisture resulted in greater loss of germination than 6 or 8 per cent but was without effect at 20° F. Seeds of high moisture contents at 40° F. or room temperature storage showed better germination after open than after sealed storage.

MATERIAL AND METHODS

The material and methods were the same as those reported in a previous paper (2). Seeds of carrot (*Daucus carota* L. var. *sativa* DC.), cauliflower (*Brassica oleracea* L. var. *botrytis* L.), eggplant (*Solanum melongena* L.), lettuce (*Lactuca sativa* L.), onion (*Allium cepa* L.), pepper (*Capsicum frutescens* L. [*C. annuum* L.]), and tomato (*Lycopersicon esculentum* Mill.) were used. The data for the four-, five-, and six-year storage periods are now available and are presented below.

In addition to the regular germination and seedling production tests, some other tests were made. It seemed of interest to determine whether old seeds would produce normal plants. For this purpose, plants were grown from six-year-old seeds and their development noted.

Further experiments were performed to ascertain whether old seeds could be removed from favorable storage conditions and packeted for some time before selling without danger of impairing their germination capacity.

Finally experiments were conducted to determine whether pre-treated lettuce seeds could be stored dry with safety before planting.

TABLE I

GERMINATION PERCENTAGES AFTER STORAGE UNDER VARIOUS CONDITIONS

Seed	Storage condition	Fresh	Stored at room temp.			Stored at −5° C.		
			4 yrs.	5 yrs.	6 yrs.	4 yrs.	5 yrs.	6 yrs.
Carrot 10.7%*	Air-dry open	67	60	53	52	66	61	59
	Air-dry sealed		0	0	0	74	64	68
	Dried over CaO to remove about 1/3 of moisture sealed		69	66	61	68	64	66
	Mixed with CaO to remove 1/3 of moisture sealed		55	66	64	68	65	63
	Mixed with CaO to remove 1/2 of moisture sealed		60	67	67	75	67	70
Eggplant 10.4%*	Air-dry open	86	77	78	74	86	77	82
	Air-dry sealed		62	72	62	84	89	84
	Dried over CaO to remove about 1/3 of moisture sealed		80	85	79	85	83	87
	Mixed with CaO to remove 1/3 of moisture sealed		76	80	81	80	85	74
	Mixed with CaO to remove 1/2 of moisture sealed		90	87	80	89	85	87
Lettuce 8.2%*	Air-dry open	98	8	0	0	91	93	94
	Air-dry sealed		0	0	0	92	93	92
	Dried over CaO to remove about 1/3 of moisture sealed		76	83	81	96	97	94
	Mixed with CaO to remove 1/3 of moisture sealed		84	77	81	97	91	93
	Mixed with CaO to remove 1/2 of moisture sealed		79	64	80	98	92	75
Onion 12.5%*	Air-dry open	98	1	1	0	97	93	89
	Air-dry sealed		0	0	0	95	94	96
	Dried over CaO to remove about 1/3 of moisture sealed		86	82	79	93	95	96
	Mixed with CaO to remove 1/3 of moisture sealed		75	62	65	97	94	97
	Mixed with CaO to remove 1/2 of moisture sealed		95	89	88	97	91	96
Pepper 10.4%*	Air-dry open	73	23	12	2	61	75	74
	Air-dry sealed		0	0	1	77	77	77
	Dried over CaO to remove about 1/3 of moisture sealed		67	30	20	78	74	69
	Mixed with CaO to remove 1/3 of moisture sealed		53	34	28	75	80	76
	Mixed with CaO to remove 1/2 of moisture sealed		63	61	53	76	69	67
Tomato 10%*	Air-dry open	93	83	81	80	91	91	94
	Air-dry sealed		61	32	28	93	95	91
	Dried over CaO to remove about 1/3 of moisture sealed		85	89	89	87	91	97
	Mixed with CaO to remove 1/3 of moisture sealed		95	89	88	90	94	89
	Mixed with CaO to remove 1/2 of moisture sealed		91	89	84	91	94	96
Cauliflower	Air-dry sealed	84	0	0	0	—	—	—
	Dried over CaO to remove about 1/3 of moisture sealed		60	43	19	—	—	—

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* Percentage of moisture (on basis of dry weight) in seeds at the beginning of the experiment.

RESULTS AND DISCUSSION

EFFECT OF STORAGE ON VIABILITY

Storage at room temperature. The results of germination tests on stored seeds are shown in Tables I and II and Figure 1. At the end of the six-year storage period the germination of all seeds had suffered a decrease under one or more storage conditions. Of all of the seeds tried, eggplant proved most resistant to deterioration showing only a slight reduction in germination capacity for lots stored air-dry either open or sealed (Table I) at room temperature. The curves for response from germination tests after storage for one to six years were similar regardless of the moisture content of the seeds at the time of storage. This indicated that these seeds were not as sensitive to moisture content as the others included in this study (Fig. 1). Although very little difference was noted in germination of seeds from open or sealed storage, air-dry seeds which had been sealed showed a consistently lower germination than those from open storage and the latter resulted in consistently lower germination than those which had been dried before sealing. It should be kept in mind that deterioration in open storage at room temperature in the vicinity of Yonkers, New York, is quite different from that to be expected in other regions with different temperature and humidity conditions. Some of these differences are reported in another section in this paper.

Tomato ranked second in keeping quality but in this case vitality was seriously impaired after five years of storage air-dry sealed. Although germination on filter paper at controlled temperature of 25° C. remained as high as 28 per cent after six years of storage, only 5 per cent of the seedlings were capable of pushing through the soil (Tables I and II). Six years of open storage also resulted in a decided decrease in seedling production from tomato seeds though the germination percentage as indicated by tests on moist filter paper remained high. In cases of both eggplant and tomato, reduction of moisture content alone sufficed to keep the seeds viable at room temperature for at least six years. It was to be expected that seeds of these two varieties would show least deterioration at this time since at the time of the previous report (after three years of storage) these were the only seeds with their viability unimpaired in all storage conditions. As the storage period lengthened, however, the beneficial effect of extra drying became apparent.

Carrot seeds lost their vitality completely in four years if air-dry seeds were sealed. Reduction in moisture content, however, extended the life of these seeds so that good germination and seedling production were obtained after four years with slight decrease but still fairly good after five years. Although germination tests after six years indicated that these seeds were still in good condition, their vigor had decreased so that seedling production in the soil had fallen off markedly (Table II).

TABLE II

SEEDLING PRODUCTION IN SOIL IN GREENHOUSE AFTER STORAGE UNDER VARIOUS CONDITIONS

Seed	Storage condition	Fresh	Stored at room temp.			Stored at −5° C.		
			4 yrs.	5 yrs.	6 yrs.	4 yrs.	5 yrs.	6 yrs.
Carrot 10.7%*	Air-dry open	54	54	37	22	63	61	43
	Air-dry sealed		0	0	0	64	48	53
	Dried over CaO to remove about 1/3 of moisture sealed		55	46	29	72	44	54
	Mixed with CaO to remove 1/3 of moisture sealed		53	44	19	63	48	30
	Mixed with CaO to remove 1/2 of moisture sealed		62	51	33	62	42	31
Eggplant 10.4%*	Air-dry open	76	73	67	63	80	68	77
	Air-dry sealed		68	44	39	79	75	78
	Dried over CaO to remove about 1/3 of moisture sealed		70	69	59	86	62	80
	Mixed with CaO to remove 1/3 of moisture sealed		69	76	69	80	78	62
	Mixed with CaO to remove 1/2 of moisture sealed		68	76	65	81	79	71
Lettuce 8.2%*	Air-dry open	13	0	0	0	22	40	76
	Air-dry sealed		0	0	0	47	53	65
	Dried over CaO to remove about 1/3 of moisture sealed		65	72	46	75	28	50
	Mixed with CaO to remove 1/3 of moisture sealed		52	56	57	38	38	39
	Mixed with CaO to remove 1/2 of moisture sealed		26	36	60	48	41	39
Onion 12.5%*	Air-dry open	92	0	0	0	87	88	78
	Air-dry sealed		0	0	0	92	90	81
	Dried over CaO to remove about 1/3 of moisture sealed		82	78	57	94	91	90
	Mixed with CaO to remove 1/3 of moisture sealed		63	50	42	94	91	86
	Mixed with CaO to remove 1/2 of moisture sealed		91	79	78	87	84	87
Pepper 10.4%*	Air-dry open	63	16	1	0	66	61	40
	Air-dry sealed		0	0	0	72	66	59
	Dried over CaO to remove about 1/3 of moisture sealed		32	4	0	73	63	54
	Mixed with CaO to remove 1/3 of moisture sealed		28	11	0	73	58	57
	Mixed with CaO to remove 1/2 of moisture sealed		59	48	14	62	57	49
Tomato 10%*	Air-dry open	82	75	68	58	78	77	67
	Air-dry sealed		46	19	5	91	69	76
	Dried over CaO to remove about 1/3 of moisture sealed		85	69	72	90	82	83
	Mixed with CaO to remove 1/3 of moisture sealed		91	71	78	89	81	65
	Mixed with CaO to remove 1/2 of moisture sealed		90	79	78	83	74	58
Cauliflower	Air-dry sealed	74	0	0	0	—	—	—
	Dried over CaO to remove about 1/3 of moisture sealed		56	19	3	—	—	—

* Percentage of moisture (on basis of dry weight) in seeds at the beginning of the experiment.

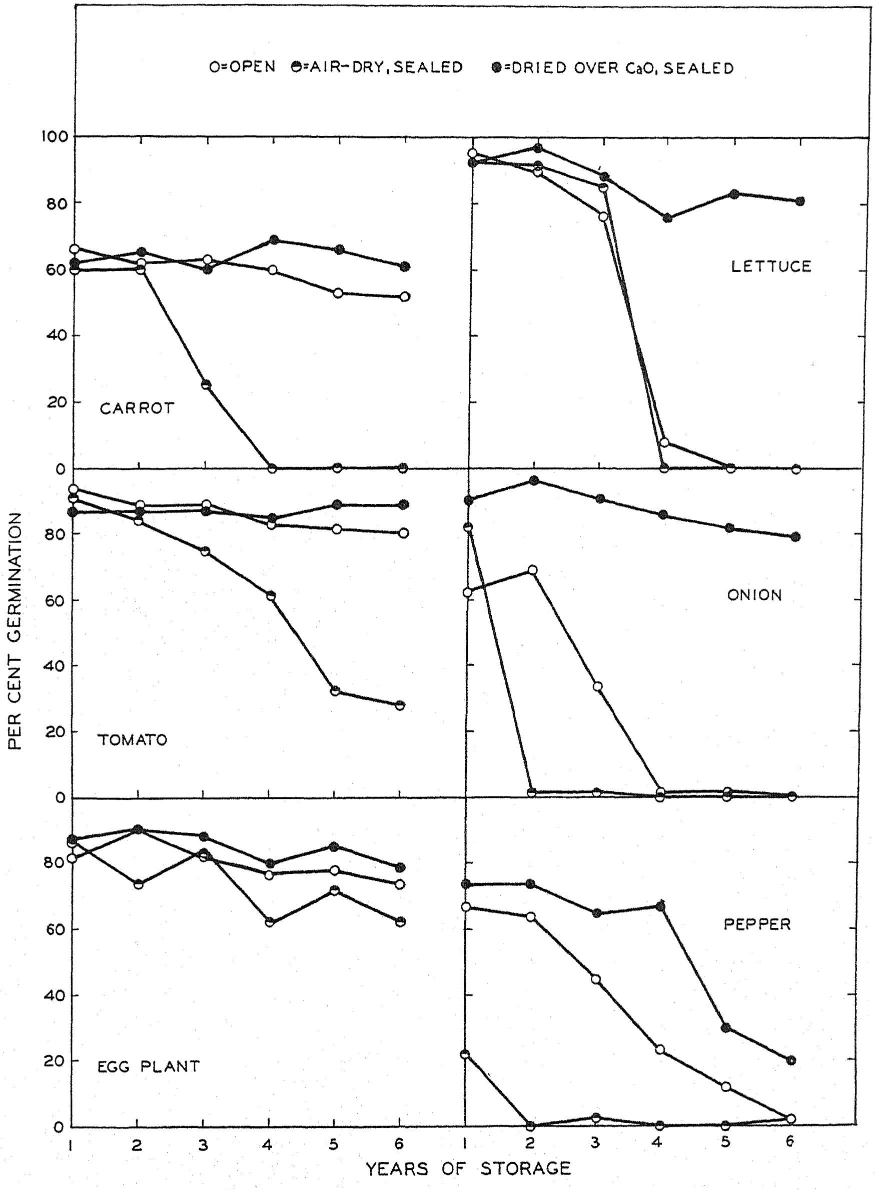

FIGURE 1. Deterioration of seeds at room temperature storage. Germination tests made in ovens on moist filter paper.

If sealed storage were used reduction of moisture content was distinctly beneficial for seeds of eggplant, tomato, and carrot, but proved only slightly superior to open storage at the six-year period.

Air-dry pepper seeds showed no germination after storage in a sealed container for two years (Fig. 1). Germination of seeds from open storage decreased less rapidly and drying delayed deterioration so that seeds were still viable after four years. However, low temperature was necessary for the preservation of these seeds for longer than four years.

For seeds of lettuce and onion, reduction in moisture content resulted in a prolonged longevity. In lettuce this effect was somewhat delayed, becoming marked only after four years of storage. Onion seeds were much more sensitive. Storage of air-dry seeds in sealed containers resulted in the death of all of the seeds before the end of the two-year period. Decrease in vitality in open storage was not quite so rapid but was significant after three years. In the case of onion the reduction in moisture content to approximately 6 per cent (one-half of moisture removed) proved better than reduction to 8 per cent (one-third of moisture removed) as evidenced by the response after four, five, and six years of storage as indicated by both soil and filter paper tests. More limited tests with cauliflower seeds indicated the same response as lettuce and onion.

Soil tests in the greenhouse exhibited consistently lower viability percentages than those made on filter paper at controlled temperatures but the relation between the two methods remained practically constant throughout. As the seeds lost their germination capacity, however, there was a tendency for the difference between results from soil and filter paper tests to widen, the seedling production in soil falling off more rapidly than germination on filter paper. There was also some indication of a reduction in speed of germination of these deteriorated seeds, the appearance of the seedlings above soil taking place two to five days later than in the case of unimpaired seeds. This delay was not evident when the seeds were placed on filter paper for germination.

To keep these vegetable seeds viable for at least six years at room temperature, then, it was necessary to adjust the moisture contents to approximately 6 to 8 per cent and place in sealed containers in order to maintain the lowered moisture. Two variations from this general rule were noted. Eggplant seeds could be kept for six years at room temperature with only slight reduction in vitality regardless of moisture content. Although the life of pepper seeds at room temperature could be lengthened by reduction in moisture, low temperature storage was necessary for maintaining germination power unimpaired.

Storage at −5° C. A temperature of approximately −5° C. permitted the maintenance of vitality for six years in all the vegetable seeds included in this study regardless of the moisture content. Seeds also kept equally well in open and sealed containers. For storage in a room below freezing for a period of six years no moisture adjustments were necessary.

EFFECT OF STORAGE FOR SHORT PERIODS AT VARIOUS HUMIDITIES AND TEMPERATURES

A practical question which often arises in regard to stored seeds is whether it is safe to remove the seeds from favorable storage conditions and packet them ready for market some time in advance of the demand for the seeds.

At the end of the five-year storage period, samples of carrot, eggplant, lettuce, onion, pepper, and tomato stored sealed air-dry and with reduced moisture at both room temperature and −5° C. as well as open at −5° C. were removed from storage and placed in manila envelopes at room temperature for one and three months, after which they were tested. The samples were removed so that seeds taken from storage conditions directly as well as those packeted for one and three months were tested at the same time. Results indicated no effect on germination from one or three months' packeting. Since the seeds were removed from storage in February and April and were tested in May, fairly cool temperatures prevailed.

TABLE III

PER CENT GERMINATION AT 25° C. AFTER STORAGE FOR MONTHS

Storage		Onion*				Pepper*			
Temp. ° C.	% relative humidity	1/2	1	2	3	1/2	1	2	3
20	Open	83	87	—	82	89	78	—	92
	20	90	87	91	90	93	93	91	92
	50	82	88	85	84	94	91	91	90
	70	68	80	68	53	85	83	76	69
	90	80	76	52	4	86	74	59	24
25	Open	79	81	—	77	86	81	—	88
	20	89	82	83	81	90	93	80	88
	50	89	66	75	—	90	92	81	—
	70	77	17	—	3	76	66	—	47
	90	53	5	0	—	83	63	4	—
30	Open	84	78	—	74	89	83	—	81
	20	84	82	86	83	92	90	91	89
	50	85	82	78	76	87	85	79	89
	70	86	59	8	0	93	73	62	18
	90	11	0	0	0	69	32	2	4

* Onion seeds gave 88 per cent and pepper seeds 82 per cent germination when experiment started.

It seemed desirable to try the effect of higher temperatures and humidities on seeds removed from storage since these conditions prevail in many places at the time for packeting. In May, 1938, certain seeds of onion and tomato from storage were placed in desiccators with approximately 43 and 93 per cent relative humidities at 25° and 35° C. Tests were made after two weeks and one, two, and three months. Tests after two weeks showed that all of the onion seeds at 93 per cent humidity were dead at both 25° and 35° C. The tomato seeds at 35° C. had all perished and only occasional seedlings were obtained from those at 25° C. No germinations were obtained from any of the 93 per cent humidity lots after one month of storage.

At 43 per cent relative humidity and 35° C. there was a drop in vitality of both onion and tomato, which had had previous unfavorable storage conditions, after two weeks of storage. This drop was also evident at 25° C. Seeds which had been under more favorable conditions of storage, however, suffered no loss of vitality when removed from storage condition and kept at 43 per cent humidity.

The response of six-year-old onion seeds to further storage at 43 per cent relative humidity at 25° and 35° C. is shown in Figure 2. Here it is clear that seeds from the most favorable storage condition (dried over CaO and stored sealed at −5° C.) were not affected by an additional three months at either 25° or 35° C. Seeds from less favorable storage conditions, however, declined rapidly in germination power at both 25° and 35° C., the loss being greater at the higher temperature in each case. There was a greater loss in seeds mixed with calcium oxide to remove one-third of the moisture than in those dried over calcium oxide to remove the same amount of moisture although both germination and seedling tests indicated that these seeds were practically equal in germination capacity when they were removed from the original storage cans. Seeds mixed with calcium oxide to remove one-half of the moisture and stored at room temperature were intermediate in their response, i.e., showed lower germination percentages than those from −5° C. storage but higher than the more moist seeds stored at room temperature (not shown in figure).

Since the quantity of seeds in storage was not sufficient for further tests a new supply of onion and pepper was obtained in April, 1938, and tests were started in which the seeds were stored in desiccators over saturated solutions of $KC_2H_3O_2$, $Ca(NO_3)_2 \cdot 4H_2O$, $NH_4Cl + KNO_3$, and $NH_4H_2PO_4$ at temperatures of 20°, 25°, and 30° C. According to Spencer (13) saturated solutions of these chemicals give relative humidities as follows:

$KC_2H_3O_2$ —20% at 20° C.
$Ca(NO_3)_2 \cdot 4H_2O$—56% at 18.5° C.; 51% at 24.5° C.
$NH_4Cl + KNO_3$—72.6% at 20° C.; 71.2% at 25° C.; 68.6% at 30° C.
$NH_4H_2PO_4$ —93.1% at 20° C.; 93% at 25° C.; 92.9% at 30° C.

Germination tests were made on moist filter paper at 25° C., using duplicates of 100 seeds each for each test. The results are shown in Table

III. Humidities of approximately 20 or 50 per cent permitted retention of vitality for at least three months at 20°, 25°, or 30° C. At 70 per cent relative humidity, however, both onion and pepper seeds deteriorated with an increased period in storage and increased temperature. Onion seeds could not be stored safely for periods longer than two weeks at 25° or 30° C. with 70 per cent relative humidity. Two months at this humidity still permitted good germination if the storage temperature was as low as 20° C. Pepper seeds were not so sensitive and could be stored at least one month at 70 per cent humidity and 25° or 30° C. temperature.

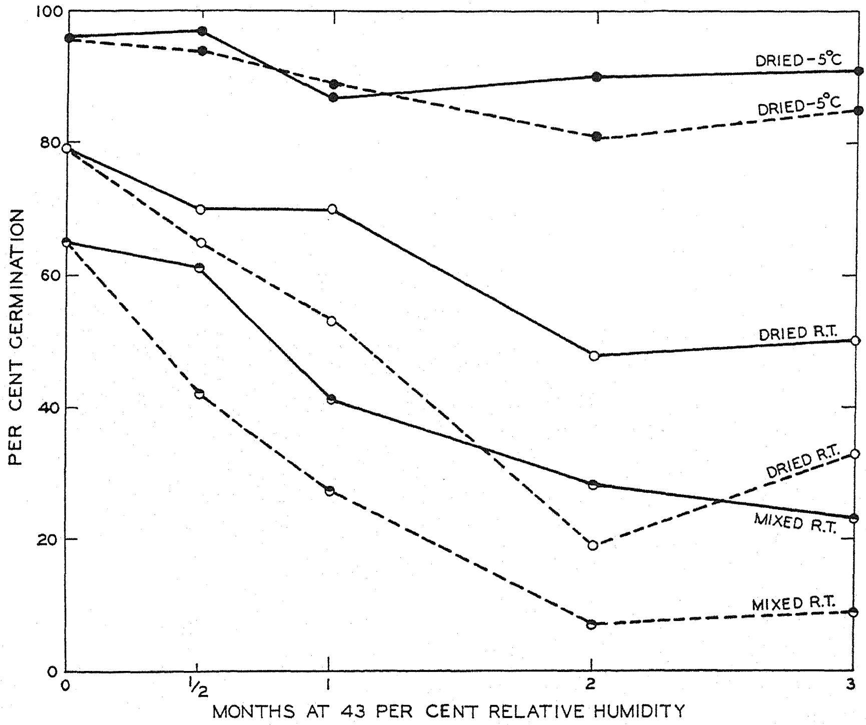

FIGURE. 2. Effect of removal of onion seed from certain storage conditions to 43 per cent relative humidity chambers. Seeds were dried over CaO and stored sealed at room temperature and −5° C.; or mixed with CaO to remove about ⅓ of moisture and stored at room temperature. Dotted lines represent further storage at 35° C., and solid lines at 25° C.

When the relative humidity was as high as 90 per cent the temperature had to be 20° C. or lower in order to keep onion seeds viable for even two weeks. Again pepper seeds proved less sensitive than onion but they also were seriously affected by high humidities and high temperatures. From these results, then, it would seem that if high humidities prevail in regions where seeds are removed from storage, it will be necessary to keep the seeds at comparatively low temperatures in order to keep them viable up to the time of sale. With humidities of 50 per cent or lower the temperature may be as high as 35° C. for seeds with high germination power and the storage period as long as three months without serious impairment of germination.

These results are in general agreement with those of Akamine and Ripperton (1) who recently reported a study of humidity and temperature effects on the viability of some crop seeds in Hawaii. No germination of lettuce seeds was obtained by these authors after 18 months in relative humidities of 75 and 90 per cent at room temperature averaging 75° F. but they kept perfectly at humidities of 60 per cent and below. They found that if the temperature could be reduced no humidity control was necessary. The occurrence of lower germination percentages in the air-dry sealed control than in the open control they attribute to a lack of circulation of air. In view of the work reported here it seems more likely that this is due to a high moisture content of the seeds. No actual moisture determinations were reported.

The sensitivity of seeds to high humidities and temperatures might explain the great variation in germination percentages obtained from seeds in commercial packets as reported by Brown and Goss (6).

STORAGE OF LETTUCE SEED AFTER PRE-TREATMENT

Different methods have been used to break the dormancy of lettuce seeds or to induce their germination at higher temperatures which would be inhibitory under ordinary conditions. Light and low temperature have been used more commonly but carbon dioxide and other chemical stimulation has also been reported (14, 15, 16). It is a matter of some interest to seedsmen and growers to determine whether these pre-treatment effects will persist if the seeds are dried before planting.

Borthwick (5) in his recommendation for pre-treatment and planting of lettuce seeds remarked that the pre-treated seeds were dried at room temperature before planting but he did not state the length of the drying period. Flint (7) reported that the light effect on dormant lettuce seeds was still evident after a period of dry storage of several weeks. Whether lettuce seed whose dormancy has been broken by light will remain non-dormant when dried depends upon the physiological condition of the seed, the period of exposure to light, and the temperature and manner of drying according to Shuck (11).

Most of these studies have been concerned with the breaking of dormancy, and not with the induction of germination at temperatures ordinarily prohibitive. Thornton (15) found the presence of carbon dioxide permitted germination at 35° C. Shuck (10), on the other hand, found that exposure to light did not overcome the natural tendency of freshly-harvested seeds to go into dormancy at 25° or 30° C. Seeds of annual delphinium may be induced to germinate at high temperatures by pre-treatment for a short period in a moist condition at low temperatures (3).

The purposes of the different temperature pre-treatments reported below were to determine whether they would induce germinations at high temperatures and whether this effect would remain after the seeds were dried. Methods of pre-treatment tried included the use of 1° C. and 5° C. for one, two, three, four, and seven days; 10° C. for one, two, and three days; 15° C. and 25° C. for two, four, six, sixteen, and twenty-four hours; and 20° C. for two, four, six, eight, ten, twelve, fourteen, sixteen, eighteen, twenty, twenty-two, and twenty-four hours. In each case the longest period represented the maximum time the seeds could be kept at the pre-treatment temperature without germination taking place.

At the end of the pre-treatment periods samples of the seeds were transferred immediately to 20° C., a favorable germination temperature, and to 3c° C., an unfavorable germination temperature. Moist filter paper was used as the germination medium. It was found that all of these pre-treatments were effective in bringing about germination at 30° C. where only 0 to 3 per cent germination was obtained without pre-treatment. The time required for pre-treatment depended on the temperature used. Four days at 1° C., four days at 5° C., three days at 10° C., one day at 15° C., eight to twenty-four hours at 20° C., and six to sixteen hours at 25° C. were best pre-treatment intervals. Of these temperatures and periods, all were equally effective in bringing about germination at 30° C. except 1° C., which was slightly inferior. This obviates the necessity for a cold chamber for pre-treatment as any temperature up to and including 25° C. can be used with success.

Samples were also placed at 35° C. for germination after having been pre-treated at 5° C. for four days and at 20° C. for fourteen, sixteen, and twenty-four hours. Thirty-five degrees C. proved too high to allow any germination without pre-treatment but after above pre-treatments germination up to 34 per cent was obtained even at this high temperature.

These pre-treatments then would be of tremendous value to growers who must plant their lettuce seed when the weather is very warm. A series of tests were performed to determine whether the pre-treated seeds could be dried and the effect still be evident.

In one series seeds pre-treated at 5° C. for four days were planted immediately and after drying for two, six, sixteen, and twenty-four hours. Germination tests were made at 20° C. and 30° C. Results indicated that the seeds could be dried for twenty-four hours without serious impairment of germination when tested at 30° C. Germination at 20° C. remained constant (above 95 per cent) throughout the tests.

Longer periods of drying were tested with seeds which had been pre-treated at 5° C. for four days, 10° C. for two days, and 20° C. for fourteen and twenty-four hours. Germination tests were made on moist filter paper at 20° C. and 30° C. immediately after pre-treatment and after one day, three days, one week, two weeks, one month, and two months drying in the laboratory. An oven at 35° C. was also used for testing immediately after pre-treatment and after one month of dry storage. Again it was shown that the vitality of these seeds was not injured by the pre-treatment and subsequent drying since germination at 20° C. remained constant throughout the tests. When tested at 30° C., however, there was a gradual decline in germination up to two months of dry storage, regardless of the pre-treatment given, thus indicating a loss of the pre-treatment effect. The initial favorable effect of pre-treatment at 20° C. was slightly superior to that at 5° C. This difference persisted throughout the tests.

Another series where seeds pre-treated at 5° C. for four days and 20° C. for sixteen hours were dried for different lengths of time up to three months indicated a more abrupt falling-off in germination capacity after one and two months of storage (Fig. 3). This was doubtless due to the fact that the experiment was begun in June and the laboratory temperature was high during the drying period while the previous experiment was begun in September and cooler temperatures prevailed. It should be noted here that pre-treated seeds dried for as long as three months showed evidence of injury as indicated by the appearance of many abnormal seedlings at 20° C. The germination percentages, however, remained high (Fig. 3). Dry seeds of the lot shown in Figure 3 gave 96, 4, and 1 per cent germination at 20°, 30°, and 35° C. respectively.

No germination was ever obtained at 40° C. with or without pre-treatment.

Results from these tests indicated that seeds may be pre-treated and then dried for at least three days after which a germination of 50 per cent can still be obtained at temperatures as high as 30° C. and 25 per cent at a temperature of 35° C. A few tests were made using soil instead of filter paper. If the soil was at room temperature at the time of planting, a higher percentage of seedlings from both dry and pre-treated seeds at 30° C. or 35° C. was obtained than was the case with filter paper. At 20° C., however, the seedling production in soil was only one-half that obtained on filter paper.

Shuck (12) found that freshly-harvested seeds which failed to germinate on blotters at 20° C. due to the high temperature germinated much better when tested in soil.

Although very few seedlings were produced when dry lettuce seeds were placed on moist filter paper at 30° C., the seeds remained imbibed and viable for considerable periods of time. Several lots of lettuce seeds (Iceberg) received April 5, 1938, were placed on moist filter paper at 30° C. and transferred to 20° C. for germination after one, two, and three weeks, and one and two months, where the germination was 95, 86, 84, 92, and 84 per cent respectively. How much longer these seeds will remain viable in a moist condition at 30° C. is being tested at the present time.

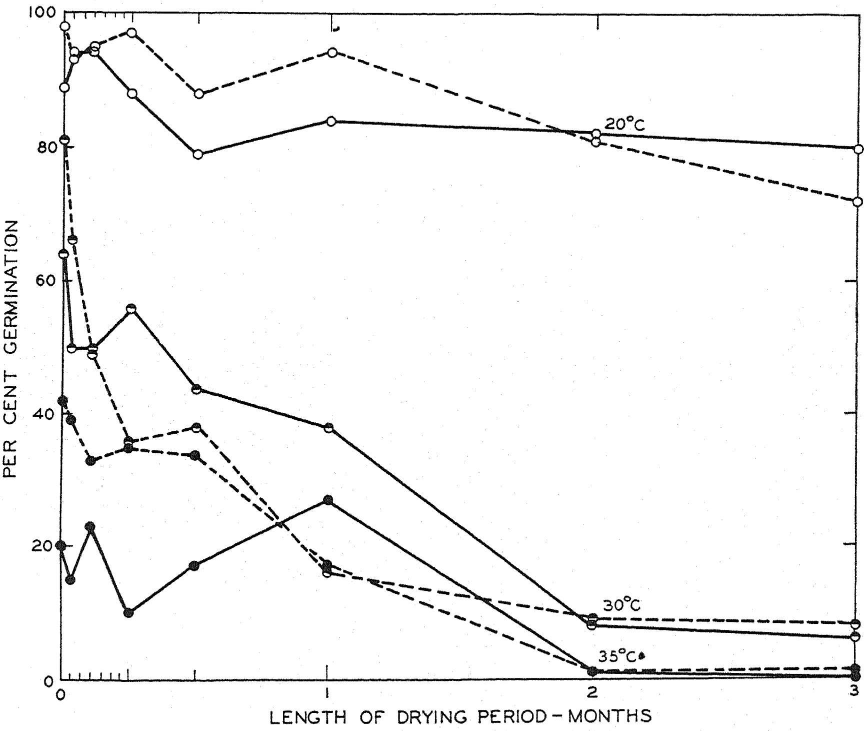

FIGURE. 3. Effect of pre-treatment and drying after pre-treatment on the germination of lettuce seed at 20°, 30°, and 35° C. Dotted lines represent pre-treatment at 20° C. for 16 hours. Solid lines represent pre-treatment at 5° C. for 4 days.

GROWTH OF SEEDLINGS FROM OLD SEEDS

In order to test whether six-year-old vegetable seeds would produce normal healthy plants, seedlings from stored seeds planted in the greenhouse on May 27, 1938, were kept and finally placed in an experimental plot outside for growth. All seedlings used for these observations were produced from seeds stored at room temperature since it was supposed that these would be more apt to exhibit irregularities of growth. Besides fresh seeds of each type which were used as controls, seeds from the following storage conditions were used:

Carrot —air-dry open; dried over CaO sealed.
Eggplant—air-dry sealed; dried over CaO sealed.
Onion —dried over CaO sealed.
Tomato —air-dry open; air-dry sealed; dried over CaO sealed.
Lettuce —dried over CaO sealed.

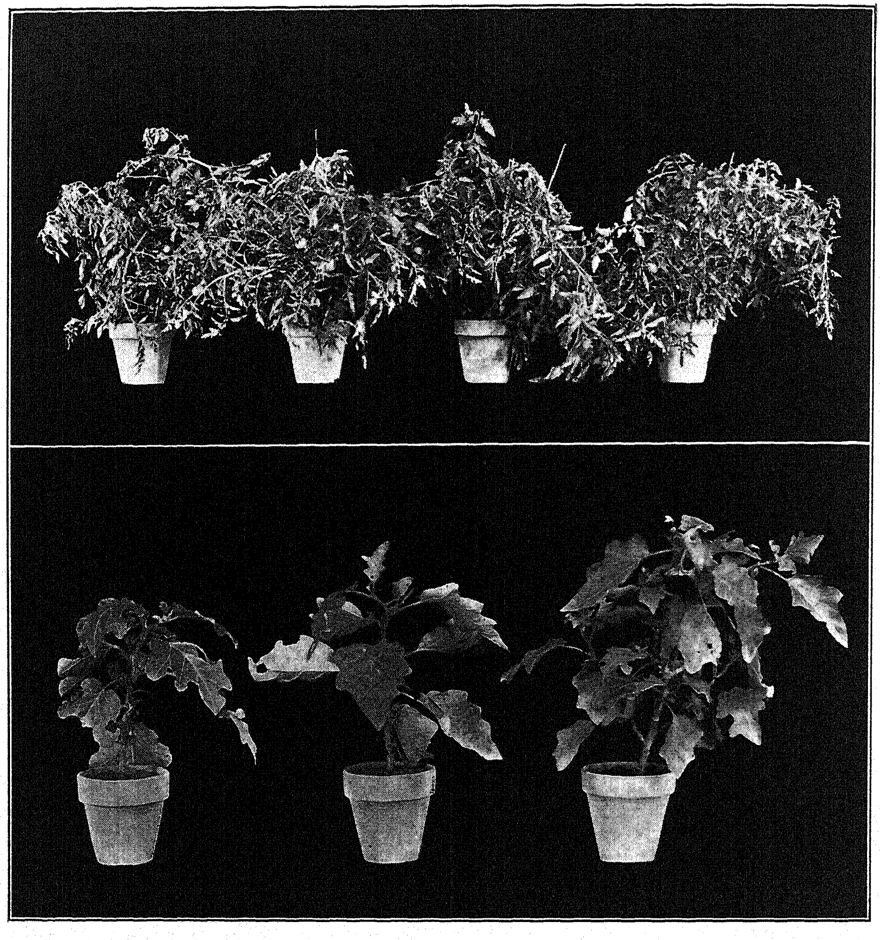

FIGURE 4. Top row, left to right: Tomato plants grown from fresh seed, and seed stored for six years at room temperature open, sealed air-dry, sealed after drying over CaO. Bottom row, left to right: Eggplant seedlings grown from fresh seed, and seed stored for six years at room temperature sealed air-dry, and after drying over CaO.

Twenty-five plants each from seeds of each storage condition and fresh seeds were observed.

Tomato plants from seeds sealed air-dry were somewhat smaller than all of the others up until July 13, after which there seemed to be no differences in growth. This is shown in Figure 4 which represents typical plants from each of the lots which were dug up, potted, brought into the studio, and photographed on August 29, 1938.

Blossoming and fruit formation occurred at about the same time and equally on all plants with a possible slight delay in plants from the air-dry sealed seed lot. It appears, then, that satisfactory growth can be obtained in plants produced from seed whose vitality as expressed by seedling production in the soil has been reduced to 19 per cent as compared with 82 per cent seedling production from fresh seeds. Six-year-old seeds from favorable storage conditions, retaining approximately 80 per cent of their original vitality, produced plants as vigorous in every respect as those produced from fresh seeds.

Eggplant seedlings showed a type of growth response similar to the tomatoes. Again air-dry sealed storage of seeds tended to retard initial growth of the seedlings, but later growth equalled that of the control plants from fresh seed. In this case also retarded growth accompanied definite decrease in seedling-producing power of the seeds (Table I). Seeds dried over calcium oxide produced plants which appeared better than the controls. This difference may have been due to the fact that these plants were in a lower place in the field and hence received a little more moisture than the other two lots. Appearance of typical seedlings is shown in Figure 4.

Onions and lettuce seedlings from the seed lots stored sealed after drying over calcium oxide were of the same general appearance and behavior as the control lots, as was also noted for carrot seedlings.

SUMMARY

The life-span of lettuce, onion, and cauliflower seeds stored at room temperature could be prolonged markedly by adjustment of moisture contents to approximately 6 to 8 per cent. Reduction in moisture content also proved beneficial for seeds of tomato and carrot if sealed containers were used at room temperature but these seeds also remained viable in open containers. Seeds of eggplant indicated a response similar to those of tomato and carrot, although the former, air-dry, remained viable much longer in sealed storage. Although reduction in moisture content delayed deterioration of pepper seeds at room temperature, results indicated that low temperature was necessary for successful storage for periods longer than four years.

Germination tests of old and fresh seeds stored for short periods at various humidities and temperatures indicated that with relative humidities of 50 per cent or lower, the temperature may be as high as 35° C. for seeds with high germination power and the storage period as long as three months without serious impairment of germination. At relative humidities above 50 per cent, however, safe storage temperatures were 20° C. or lower. This is of practical importance in commercial packeting of seeds after removal from favorable storage conditions.

Pre-treatment of lettuce seeds on a moist medium at 25° C. or below permits germination at high temperatures which are ordinarily prohibitive. Pre-treated seeds may be dried at room temperature for at least three days after which a germination of 50 per cent can still be obtained at 30° C., and 25 per cent at a temperature of 35° C.

Seedlings of carrot, eggplant, onion, tomato, and lettuce grown from six-year-old seeds which had been stored under favorable conditions for retention of vitality showed normal vigor in further growth. A slight initial retardation of growth was noted in cases of unfavorable storage conditions.

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STORAGE OF ELM SEEDS

LELA V. BARTON

INTRODUCTION

Retention of vitality of certain seeds which normally live but a short time in open air storage is a subject of economic as well as of scientific interest. Among seeds belonging to this group are those of the American elm (*Ulmus americana* L.). It has been commonly supposed that these seeds must be planted as soon as they mature in early summer with consequent impairment of seedling stands by the drought and heat of summer and the necessity for the protection of the tender young plants with the beginning of cold weather in the fall.

Steinbauer and Steinbauer (5) desiccated elm seeds over different concentrations of sulphuric acid for a period of two weeks, after which they placed them in sealed containers at 0°, 10°, and 20° C. They found that low temperature and low water content were favorable for retention of vitality, the seeds remaining good up to nine months of storage and degenerating rapidly thereafter. Germination was much reduced after eleven months, which is the approximate time fruits would have to be stored to be available for nursery planting in many localities. Their germination tests were made between moistened blotters at 20° C. No soil tests were reported. They also noted that elm seeds have a rest period regardless of storage conditions but influenced both by the moisture content of the seeds and by storage temperature. No actual moisture determinations were made.

Delayed germination in American elm has also been reported by Rudolf (4), who obtained new seedlings in May, 1937 from seeds planted in June, 1936. He attributed this delay to extraordinary meteorological factors which also made possible the retention of vitality for one year.

George (2) stored American elm seeds in containers with tight-fitting covers placed in an attic of an unheated building. Outdoor temperatures ranged from 115° F. to −28° F. during the period of the test. Since he obtained 79 per cent germination on moist blotters at room temperature after 10-1/2 months of storage, he concluded that no special storage condition was necessary. Soil tests of these seeds yielded 33 per cent seedling production.

Manaresi (3) found that the germinative capacity of the English elm (*Ulmus campestris* L.) remained fairly constant for two months and then diminished so that practically all of the seeds were dead after six months. He also stated that part of the fresh seeds did not germinate promptly but were more or less retarded up to a maximum of twenty-two days. This retardation, he said, depended on the "embryonic post-maturation."

MATERIAL AND METHODS

Before storage experiments were begun, samples of intact fruits as well as seeds with the fruit coats removed were placed in moist granulated peat moss at constant temperatures of 15°, 20°, 25°, and 30° C., and daily alternating temperatures of 10° to 20° C., 10° to 25° C., 10° to 30° C., 15° to 30° C., and 20° to 30° C. in order to determine the requirements for germination. It was found that daily alternations of temperature (except 20° to 30° C. which was too high) resulted in consistently better germination than any of the constant temperatures tried. In cases of alternating temperatures, the cultures were left at the higher of the two for eight hours and at the lower for sixteen hours daily. Constant temperatures of 15°, 20°, and 25° C. were effective, however. Thirty degrees C. inhibited germination. A daily alternation of 10° to 25° C. was chosen as a suitable temperature for vitality tests. The greenhouse temperature used was 21° C. except for the tests made in May, June, and August when the temperature could not be controlled. Further experiments on the germination requirements of these fruits also showed that although pre-treatment failed to increase the germination at 10° to 25° C. daily alternation very much better seedling production was obtained in the greenhouse if they were pre-treated in moist granulated peat moss for one month prior to planting. Temperatures of 1°, 5°, 10°, and 15° C. were found effective for this pretreatment. Comparative results are shown in Figure 1 A. These fruits were planted in July and the very small number of seedlings from the dry lot was doubtless due to the high temperature. Delayed germination as reported by Rudolf (4) could well have been a normal response to low temperature pre-treatment since additional seedlings were obtained after a winter outside. Plantings made in October, 1936 by George (2) also exhibited this effect since few seedlings appeared in 1936, but an excellent stand was obtained in 1937.

It was also found that soaking the seeds in water for 24 hours with simultaneous exposure to a constant light source resulted in a significant increase both in speed and percentage of seedling production. Soaking periods of six, sixteen, twenty-four, and forty-eight hours under the conditions of this experiment were equally effective in increasing seedling production. After forty-eight hours, fruits left in water started to deteriorate while a two-hour soaking period was only slightly beneficial. Soaking in the absence of light also increased germination but was less effective than when light was present. This latter method, however, proved to be inferior to low temperature for pre-treatment.

Moisture determinations were made at the time the fruits were stored by drying the samples to a constant weight in a vacuum oven at 80° C. and are expressed as percentages of the wet weight of the fruits. A period of forty-eight hours was sufficient for the drying process although weighings were usually continued over a period of three to five days. Later tests were not made.

Lot I was collected on May 24, 1937, in Yonkers, New York, two days after a heavy rainstorm had washed large lots of seeds into a gutter. Some were spread out to dry at room temperature and were stored under various conditions on June 2. Others were placed in a desiccator over calcium oxide at room temperature on May 28 and allowed to remain there for six days at which time they were removed and stored under various conditions. The air-dry fruits contained 8 per cent moisture at the time of storage while those stored in a desiccator for six days contained 7 per cent. Sealed storage of these fruits was effected by the use of distillation flasks hermetically sealed. Some of the flasks were sealed with air inside. In others the air was displaced by oxygen while still others were exhausted of air before sealing. Vacuum was obtained in the flasks by means of an oil exhaust pump and approximated one mm. or less of pressure. Enough fruits were sealed in each flask to permit viability tests for one period. Since a new flask was used for each testing period, the seeds were exposed to constant storage conditions up to the time of testing.

Lot II was collected on Pelham Parkway, Bronx, New York, on May 25, 1937, and some were spread out to dry in the laboratory and others were placed over calcium oxide in desiccators after having the moisture content ascertained. Air-dry seeds of this lot contained approximately 7 per cent moisture. It was desired to remove about one-half and three-fourths of this moisture in order to determine moisture effects on keeping quality. This was done by weighing the lots at the time they were placed in the desiccators, and then weighing at intervals subsequently until the approximate desired weight loss was obtained. The seeds were then removed and, after weighing samples were taken, were placed in sealed containers. Actual moisture determinations of these lots at the time of storage, June 25 and July 12, showed moisture contents of 3 per cent and 2 per cent respectively. Sealed storage of seeds of Lot II was in tin cans with tight-fitting lids sealed with sealing wax, care being taken to prevent heating during the sealing process. These cans were opened and a sample removed at each testing period and then re-sealed. Storage of both lots was at room temperature, at 5° C., and at approximately −5° C.

Germination tests to determine viability were made after one, four, seven, ten, and sixteen months. At each testing period fruits were mixed with moist granulated peat moss and placed at controlled daily alternation of temperature of 10° to 25° C. Germination counts were made when the radicle had appeared and had begun to elongate.

In addition to the oven tests fruits were planted in soil in the greenhouse using a mixture of equal parts of sand, sod soil, and granulated peat moss. Fruit samples were planted directly from the storage containers. Other samples were placed in bottles in water and allowed to soak for twenty-four hours exposed to light, the source of which was a sixty-watt Mazda lamp at a distance of one foot above the bottles. After soaking, the water was drained off and the seeds were planted in soil. Still other samples were mixed with moist granulated peat moss and placed at a constant temperature of 5° C. for one month prior to planting in the greenhouse. This method was shown to improve the seedling production markedly although elm seeds are not commonly supposed to require low temperature pre-treatment. Seedling production was recorded when the seedlings had appeared above the soil. One hundred seeds were used for each vitality test.

RESULTS AND DISCUSSION

From a study of Tables I and II it becomes clear that a true vitality test of elm seeds required either a favorable germination temperature (10° to 25° C. daily alternation used here) or pre-treatment by a period at low temperature or by soaking. A comparison of seedling production in the greenhouse with and without pre-treatment is shown graphically in Figure 3. Seeds pre-treated by soaking were intermediate in their seedling yield between those planted dry and those pre-treated at low temperature. These results remained uniform throughout the tests. The percentage germination at 10° to 25° C. daily alternation was much higher than that obtained from dry fruits planted in soil. However, if fruits were planted in soil after proper pre-treatment their seedling yield compared favorably with that obtained at controlled temperatures. Fruits planted dry in soil reached the maximum seedling production in approximately fourteen days. Those soaked for twenty-four hours germinated more quickly, few seedlings appearing after ten days, while those pre-treated at 5° C. for one month practically completed their seedling production within six days after planting.

About twenty days were required for complete germination at 10° to 25° C. daily alternation. The number of radicles which emerged within the first ten or twelve days corresponded fairly closely with the seedling production from dry fruits planted in soil. Hence, there seemed to be a correlation between the speed of germination at controlled temperature and the ability to push above the soil when no pre-treatment was given.

These facts should be borne in mind when fruits are tested for planting on a large scale.

Some idea of the appearance of flats of seedlings from fruits planted dry and after soaking can be obtained from Figure 1 B.

TABLE I

Lot I. Results of Vitality Tests of Stored Fruits

Moisture content	Storage		% germination or seedling production after storage for months (approx.)																			
			1 (Planted in July)				4 (Planted in Nov.)				7 (Planted in Jan.)				10 (Planted in Apr.)				16 (Planted in Oct.)			
			10° to 25° C.	GH. 1	GH. 2	GH. 3	10° to 25° C.	GH. 1	GH. 2	GH. 3	10° to 25° C.	GH. 1	GH. 2	GH. 3	10° to 25° C.	GH. 1	GH. 2	GH. 3	10° to 25° C.	GH. 1	GH. 2	GH. 3
Air-dry (8%)	Room temp.	Open	67	9	26	46	42	7	6	40	43	5	4	53	24	5	4	42	4	0	0	1
		Sealed Air	71	13	24	78	52	36	22	57	18	4	0	6	—	1	0	2	0	0	0	0
		Vac.	77	10	20	91	70	34	58	75	61	17	19	87*	45	12	8	79	44	14	26	65
		O_2	57	12	10	79	32	4	8	21	0	0	0	0	0	0	1	0	0	0	0	0
	5° C.	Open	72	10	34	82	43	8	17	30	11	3	13	82*	6	9	0	10	1	0	0	1
		Sealed Air	79	11	43	92	52	46	54	65	76	7	64	92	59	9	12	92	67	25	57	82
		Vac.	79	8	39	88	79	32	61	81	78	11	66	82	66	31	26	77	61	37	58	83
		O_2	75	9	33	89	73	30	45	83	71	13	55	78	71	18	15	88	71	27	62	70
	−5° C.	Open	75	19	38	76	71	34	72	80	76	12	6	75	84	6	10	91	74	30	45	82
		Sealed Air	73	25	43	87	79	48	62	84	81	10	31	78	76	12	14	82	70	30	48	75
		Vac.	83	19	32	84	75	20	76	84	77	22	22	84	67	14	15	82	69	36	48	81
		O_2	81	6	39	85	81	38	71	80	78	32	35	85	—	10	11	81	50	20	48	83
Dried over CaO 6 days (7%)	Room temp.	Sealed Air	75	15	45	95	54	8	45	61	55	5	4	50	43	7	0	39	24	0	3	8
		Vac.	78	8	48	89	71	35	67	75	35	1	2	31	90	14	25	69	66	14	32	76
		O_2	74	8	43	78	45	6	18	26	34	3	1	19	16	0	3	5	10	0	0	0
	5° C.	Sealed Air	88	3	33	78	86	27	43	86	76	7	56	82	69	6	14	78	69	28	48	72
		Vac.	87	5	47	90	78	37	47	85	77	13	60	92	76	8	14	89	66	20	63	81
		O_2	86	4	45	87	78	36	35	72	90	18	47	90	72	15	8	89	70	22	52	85
	−5° C.	Sealed Air	79	2	53	91	83	31	71	72	81	17	27	93*	74	10	17	86	68	34	49	85
		Vac.	78	2	48	84	82	26	68	90	78	16	28	87	79	9	9	76	75	23	61	84
		O_2	80	1	37	86	83	27	72	87	83	8	22	85	61	10	15	82	68	22	59	90

GH. 1 = Dry fruits planted in greenhouse flat without pre-treatment.
GH. 2 = Fruits planted in greenhouse flat after soaking in water under light.
GH. 3 = Fruits planted in greenhouse flat after pre-treatment for one month at 5° C.
* Many seeds rotted at 5° C., hence sample reduced in number and composed of good seeds only.

TABLE II

LOT I. COMPARISON OF GREENHOUSE AND EXCISED EMBRYO TESTS OF STORED SEEDS; PER CENT SEEDLING PRODUCTION OR GOOD EMBRYOS

Storage		Air-dry after storage for months						Reduced moisture after storage for months					
		7			10			7			10		
		GH. 1	GH. 3	Good embryos	GH. 1	GH. 3	Good embryos	GH. 1	GH. 3	Good embryos	GH. 1	GH. 3	Good embryos
Room temp.	Open	—	—	—	5	42	24	—	—	—	—	—	—
	Sealed Air	4	6	0	1	2	0	5	50	68	7	39	46**
	Sealed Vac.	17	87*	54	12	79	60	1	31	38**	14	69	54
	Sealed O_2	0	0	0	0	0	0	3	19	64**	0	5	60**
5° C.	Open	—	—	—	9	10	2**	—	—	—	—	—	—
	Sealed Air	7	92	88	9	92	92	7	82	94	6	78	94
	Sealed Vac.	11	82	86	31	77	88	13	92	96	8	89	88
	Sealed O_2	13	78	86	18	88	88	18	90	94	15	89	92
−5° C.	Open	—	—	—	6	91	84	—	—	—	—	—	—
	Sealed Air	10	78	84	12	82	82	17	93*	88	10	86	96
	Sealed Vac.	22	84	72	14	82	84	16	87	80	9	76	84
	Sealed O_2	32	85	96	10	81	92	8	85	92	10	82	94

GH. 1 = Dry fruits planted in greenhouse without pre-treatment.
GH. 3 = Fruits planted in greenhouse after pre-treatment for one month at 5° C.
* Many seeds rotted at 5° C. Only good ones planted.
** Feeble growth.

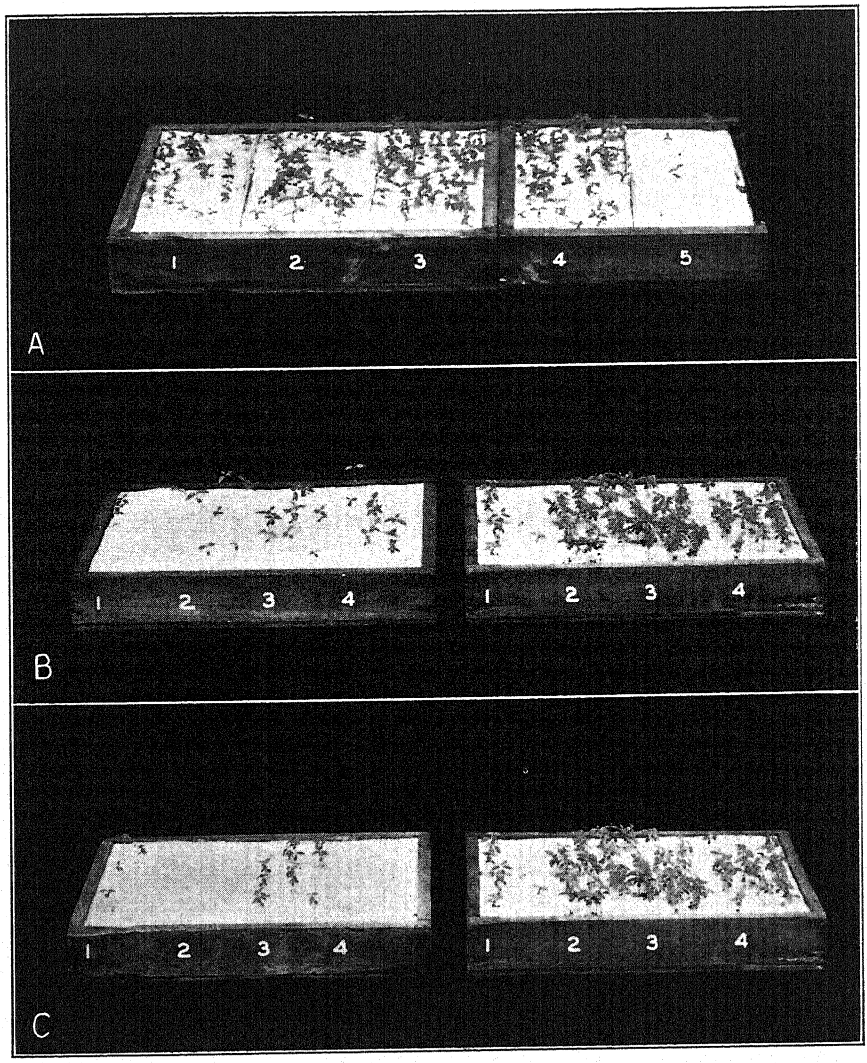

FIGURE 1. A. Effect of low temperature pre-treatment in moist granulated peat moss for one month. 1, 2, 3, 4. Pre-treated at 1°, 5°, 10°, and 15° C.; 5. Dry seeds. B. Fruits of Lot I stored at 5° C. for 7 months. Flat on left, fruits planted dry; flat on right, fruits soaked 24 hours before planting. 1. Open storage; 2, 3, 4. Sealed in air, vacuum, and oxygen. C. Fruits of Lot I stored for 7 months at room temperature (left) and 5° C. (right). Seeds soaked before planting. 1. Open storage; 2, 3, 4. Sealed in air, vacuum, and oxygen.

LOT I

Air-Dry Fruits

Storage at room temperature. Fresh fruits of this lot gave 81 per cent germination at 10° to 25° C. daily alternation and 15, 53, and 83 per cent seedling production in the greenhouse when planted dry, and after 24 hours soaking and one month pre-treatment at 5° C. These tests were made in June, 1937. The second test was made in July, 1937, when similar results were obtained (Table I). This test, however, indicated a tendency for fruits stored open at room temperature to lose some of their seedling-producing power. This decreased power was maintained by these fruits up to the tenth month of storage but none were viable after storage for sixteen months. Air-dry fruits stored at room temperature with approximately 8 per cent of moisture when sealed in air were slightly superior to those stored open up to four months of storage after which degeneration was very rapid. Those stored in an atmosphere of oxygen lost their vitality even more rapidly. The favorable effect of a vacuum was evidenced from tests up to at least sixteen months of storage, when the last test was made.

Results from the sample planting made after seven months of storage are shown in Figure 1 C. The flat on the left indicates the favorable effect of vacuum, when other storage conditions are unfavorable.

Storage at 5° C. Open storage at this temperature proved no better than open storage at room temperature although 5° C. has usually been found good for storage. The difficulty in this case was encountered in the moisture-laden atmosphere of the 5° C. room. Moisture determinations of fruits in open storage for sixteen months in this room revealed 24 per cent moisture as compared with 7 per cent in those from open storage for the same length of time at room temperature. That 5° C. was a favorable storage temperature when the moisture content was not so high was borne out by the fact that fruits in sealed storage at 5° C. whether they were sealed in oxygen, in air, or in a vacuum were capable of maintaining their germination power up to at least sixteen months of storage. The vacuum effect was not evidenced or was at least delayed when low temperature storage was used.

Storage at −5° C. By referring to Table I, one can see that a temperature below freezing was sufficient to maintain the vitality of the elm fruits regardless of the other conditions. The low temperature permitted the preservation of fruits in open containers in spite of the 14 per cent moisture which was present after sixteen months of storage.

The comparative germination capacities of fruits stored in open containers and in sealed tubes containing air together with storage temperature effects are shown in Figure 2.

Fruits Dried over Calcium Oxide

Although these fruits were stored in a desiccator over quicklime for six days prior to storage, their moisture content was reduced only 1 per cent. This seems to have been sufficient, however, to permit the seeds sealed in air or in oxygen to remain viable much longer than air-dry seeds (Table I). Here again the favorable influence of the vacuum as well as the deleterious effect of the oxygen became evident though less rapidly than in the more moist fruits. As in the case of the air-dry fruits, those with slightly less moisture kept well when sealed and placed at 5° C. or −5° C.

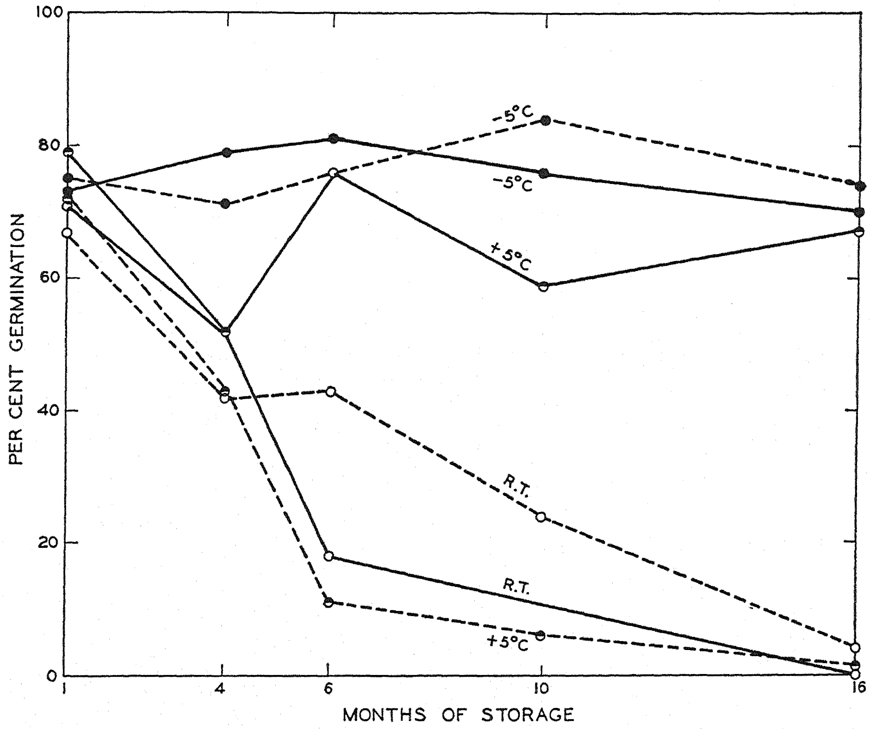

FIGURE 2. Lot I. Effect of storage under various conditions on germination at 10° to 25° C. daily alternation. Dotted lines indicate open storage; solid lines sealed storage. R.T. = room temperature.

Excised Embryo Tests

The excised embryo test for viability (1) was applied to elm fruits which had been stored for seven and ten months. The dry fruit coats were removed by gentle rubbing in cheesecloth. The seeds were then soaked in

TABLE III

LOT II. RESULTS OF VITALITY TESTS OF STORED FRUITS

Storage			% germination or seedling production after storage for months																			
			1 (Planted in Aug.)				4 (Planted in Oct.)				6 (Planted in Jan.)				10 (Planted in May)				16 (Planted in Oct.)			
Temp.	Moisture content	Open or sealed	10° to 25° C.	GH. 1	GH. 2	GH. 3	10° to 25° C.	GH. 1	GH. 2	GH. 3	10° to 25° C.	GH. 1	GH. 2	GH. 3	10° to 25° C.	GH. 1	GH. 2	GH. 3	10° to 25° C.	GH. 1	GH. 2	GH. 3
Room	7% (air-dry)	O	82	14	57	88	60	13	61	74	64	11	25	68	67	14	35	72	8	1	4	33
		S	86	16	74	81	81	29	81	93	85	29	67	97	86	32	71	91	64	43	62	79
	3%	S	82	11	74	81	86	25	81	92	85	41	78	96	81	42	73	92	75	41	63	87
	2%	S	83	12	72	87	80	33	88	88	80	54	70	89	84	39	75	82	78	27	39	54
5° C.	7% (air-dry)	O	84	13	65	83	62	29	54	59	44	6	37	70	13	4	9	13	3	1	4	0
		S	87	16	73	92	81	29	81	92	77	25	85	85	91	20	76	91	79	68	64	88
	3%	S	85	13	67	83	75	16	85	82	85	39	85	91	85	26	70	83	83	68	70	80
	2%	S	82	25	70	87	90	31	73	95	93	21	70	87	82	18	66	95	82	61	63	71
−5° C.	7% (air-dry)	O	87	16	73	79	80	64	68	91	84	46	78	95	95	37	75	87	78	72	71	80
		S	91	26	80	83	87	57	89	97	81	54	82	95	77	20	70	84	84	67	81	74
	3%	S	89	20	71	79	84	48	88	92	85	38	76	89	88	21	66	90	79	81	75	73
	2%	S	79	20	72	82	85	68	79	91	80	68	63	93	81	25	74	88	84	76	73	67

GH. 1 = Dry fruits planted in greenhouse without pre-treatment.
GH. 2 = Fruits planted in greenhouse after soaking in water under light.
GH. 3 = Fruits planted in greenhouse after pre-treatment for one month at 5° C.

water overnight and the remaining coats removed. The embryos were placed on moist filter paper in petri dishes at room temperature and examined daily. Growth of the good embryos began almost immediately so that most of them had elongated considerably and the cotyledons had turned green within three days. Some of the embryos showed feeble growth as compared with the others, but would have appeared good without better seedlings for comparison. This type of growth was rare, however, the majority of the embryos either showing vigorous growth or rotting without elongation or greening. Fifty embryos were used for each test.

The comparative results of these tests may be seen in Table II. Again it is evident that, as in the case of the controlled temperature tests, the excised embryo test cannot be expected to indicate the seedling production which would be obtained if the elm fruits were planted in soil without pre-treatment. However, when the fruits were pre-treated at 5° C. for one month and then planted in soil, the numbers of seedlings produced agreed closely in value with that expected from the results of the excised embryo tests. A few exceptions were to be found in which the excised embryos showed feeble growth as compared with other good embryos; nevertheless, these still appeared healthy. In these cases seedling production did not measure up to the expected percentages.

LOT II

This collection of fruits exhibited greater germination vigor than Lot I. This character was noticeable throughout the tests, especially as regarded seedling production in soil without pre-treatment (Table III). Fresh fruits of Lot II produced 42 per cent seedlings in soil without pre-treatment as compared with 15 per cent for Lot I. After soaking, fresh fruits of Lot II gave 80 per cent seedling production and after pre-treatment for one month at 5° C., the seedling production was 96 per cent. Germination at 10° to 25° C. daily alternation was 89 per cent and was complete two or three days sooner than for Lot I. Germination at 10° to 25° C. after six or seven days could be taken as an indication of the percentage seedling production to be expected in soil without pre-treatment.

Sealed storage of this lot was effected by using tin cans with tightly-fitting lids sealed with sealing wax. The entire lot was opened each time a vitality test was made and re-sealed after the sample was removed. In Lot I, the individual samples were sealed separately so that each one remained hermetically sealed from the time of storage to testing.

Neither vacuum nor oxygen atmosphere was used in this case, the main purpose being to test the effect of further drying. Air-dry fruits of this lot contained approximately 7 per cent moisture. These as well as other fruits with moisture contents adjusted to approximately 3 per cent and 2 per cent were stored at room temperature and at 5° and $-5°$ C.

Different storage effects are shown in Table III and Figure 3. It should be kept in mind that air-dry seeds of this lot are comparable in moisture content with those of Lot I dried over calcium oxide for six days. Fruits of Lot II maintained their vitality better than those of Lot I when those of comparable moisture contents were sealed in air. Whereas those of Lot I were capable of producing only a few seedlings after 16 months' storage, those of Lot II suffered only a slight reduction in germinating power in the same length of time. It will be noted also that fruits of Lot II lived longer at open room temperature storage than those of Lot I. Both lots behaved similarly at 5° and −5° C. storage.

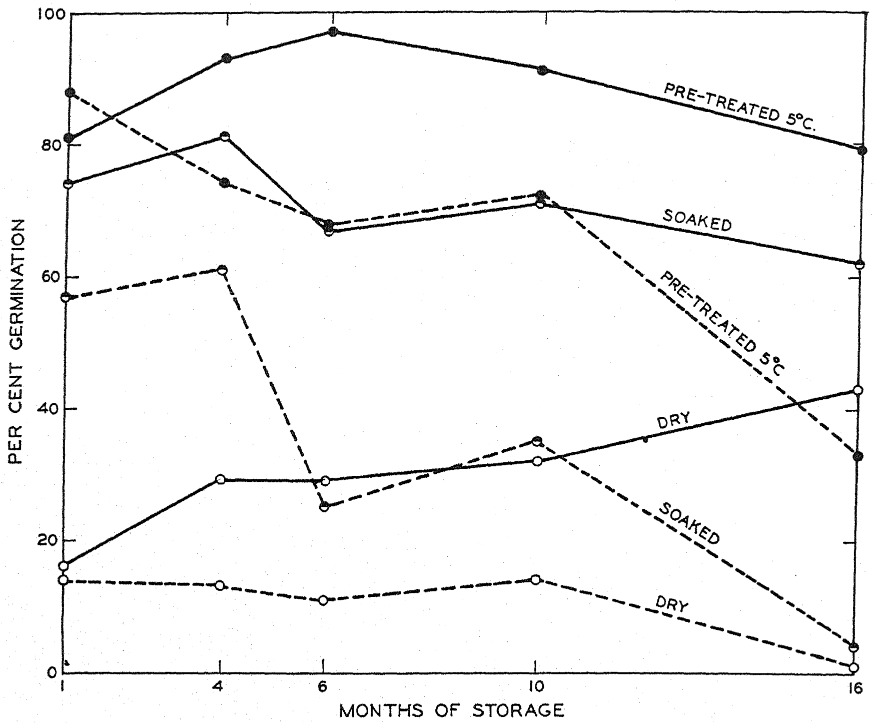

FIGURE 3. Lot II. Effect of pre-treatment on seedling production in the soil after storage at room temperature. Dotted lines indicate open storage; solid lines sealed storage.

Reduction of moisture content from 7 per cent (air-dry) to 3 per cent or 2 per cent appeared not to have harmed the fruits but no benefit was evident from drying up to sixteen months of storage.

Data obtained in these tests show little if any evidence of after-ripening in dry storage as reported by Steinbauer and Steinbauer (5). When a favorable germination temperature (10° to 25° C. daily alternation) was applied to Lots I and II no increase in germination was noted with increased length of time in dry storage (Tables I and III). Lot I exhibited increase in seedling production from dry fruits planted in soil after four months of dry storage over those planted fresh or after one month of storage but since the germination of elm fruits was shown to be inhibited by high temperatures and since the initial and one-month tests were made in June and July while the four-month test was made in November, this increase was to be expected. Subsequent decreases after the four-month high might be attributed to loss in germination vigor. A similar explanation might be applied to the results obtained from Lot II. Seeds properly pre-treated for seedling production in the soil failed to show after-ripening with dry storage.

SUMMARY

Elm fruits germinated well in moist granulated peat moss at controlled constant temperatures of 15°, 20°, and 25° C. or at daily alternating temperatures of 10° to 20° C., 10° to 25° C., 10° to 30° C., and 15° to 30° C. No pre-treatment was necessary under these conditions. However, pre-soaking in water for twenty-four hours as well as pre-treatment in moist granulated peat moss at 5° C. for one month resulted in greatly increased seedling stands in soil in the greenhouse.

Seeds with a moisture content of seven per cent proved superior in germination capacity to those with eight per cent after storage in sealed containers at room temperature. An atmosphere of oxygen proved especially deleterious when both moisture content and storage temperature were relatively high. A vacuum served to prolong vitality in cases in which other storage conditions were unfavorable.

Elm seeds can be kept viable for at least sixteen months by sealing in containers kept at 5° C. or below.

Different seed lots vary both in initial germination capacity and in keeping quality under various conditions.

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GERMINATION AND GROWTH OF SOME ROCK GARDEN PLANTS

Eltora M. Schroeder and Lela V. Barton

INTRODUCTION

In recent years there has been a growing consciousness regarding the beauty and desirability of rock gardens and with it an increase in the number of requests for information which is "more accurate than the present trial and fail method" of cultivation. The experiment reported here gives the results of germination tests made on seventeen species of rock garden and alpine seeds which have been among the trouble makers.

Literature on rock garden and alpine species is quite abundant but for the most part very general, dealing with genera rather than with species. Ingwersen's (17) statement, that hard and fast rules cannot be laid down for alpine seeds since they differ too much, sums up the situation very well. In spite of this statement, he does give a few general rules: Sow the seeds soon after ripening, expose them to as severe cold as possible, and do not cover them too deeply. Pearce (26) recommends late autumn and early winter sowing for *Primula*, *Gentiana*, *Pentstemon*, *Calochortus*, *Camassia*, *Cytisus*, *Lewisia*, and *Meconopsis*. DeBevoise (7) groups the alpines into one class with instructions to place newly collected seeds in a refrigerator for a few weeks' treatment and after the first frost, to plant outside in a well-drained soil. He adds that they do not always germinate the first season. Placing the soil over a layer of stones in a pan or scattering the seeds in moss from the woods are methods which may also be used. McCully (19) gives practically the same method for slow-germinating alpines. Correvon (6) states that a light sandy soil, sufficiently nourishing for the young plants, is necessary. The best season for sowing alpine seeds is late November, early December, or, where snow is deficient, in the spring. Abundant drainage in a cool, dry, clean frame is necessary. If, however, snow should fall, the seeds should be covered with a thick layer of it and water sprayed on top, so that it may freeze over. This assures a better stand of seedlings than when the pots are left uncovered. Archie (3) and Amsler and Lawrence (1) also have found freezing to be good for hardy *Primula*, *Meconopsis*, *Gentiana*, and, in fact, for all true alpines. Alpine seeds have been divided into four classes by Malmo (21) according to the time of planting. Hard-shelled alpine seeds should be sown in late fall, notoriously difficult seeds in mid-winter, others in late winter, and the seeds from mild climates in early spring.

Sand, loam, and leaf mold, peat or humus, usually in equal parts, are the soil mixtures suggested in the literature (2, 3, 6, 7, 17, 19, 23).

Lewisia plants require a moist loamy soil, richly prepared, sharply drained, and rocky underneath (10). They should be kept very wet during the growing period, but in order to protect the neck of the plant it should be surrounded by chips. Neeman (24) filled a box five or six inches deep, half full of common peat or sphagnum. Over this he placed a mixture of one-third leaf mold and two-thirds sharp clean sand leaving a space about one inch in depth. He covered this with a layer of the same soil very finely sifted, filling the box to within one-fourth inch of the top. He watered the seeds from the bottom.

Ebner (8) reports that *Draba verna* fluctuates in vitality with good response in September and March and poor results in winter and in May.

The present paper is a report of a series of experiments designed to obtain more definite information on the germination and growth requirements of a number of these genera.

MATERIALS AND METHODS

The seeds for these experiments were obtained from John Abercrombie and Fred Perry, Vancouver, Canada, Fred Gibson, Boyce Thompson Southwestern Arboretum, Superior, Arizona, Mrs. Charles King, Larchmont, New York, Mrs. G. R. Marriage, Upton Gardens, Colorado Springs, Colorado, and Somerset Rose Nursery, New Brunswick, New Jersey. The following species were included in the experiment begun in 1937: *Calochortus macrocarpus* Dougl., *Camassia leichtlinii* Wats., *Campanula barbata* L., *C. garganica* Tenore, *Cytisus decumbens* Spach, *Draba aizoides* L., *D. alpina* L., *Gentiana lagodechiana* Kusn., *Hypericum coris* L., *Lewisia rediviva* Pursh, *Meconopsis cambrica* Vig., *Mimulus langsdorfii* Donn, *Pentstemon ambiguus* Torr., *Primula denticulata* Sm., *P. obconica* Hance, *P. pulverulenta* Duthie, and *Ramondia pyrenaica* Rich. These species were studied because tests made on seeds sent to us previously had not been successful or comprehensive enough, or requests concerning them had been received here. Results of some of the earlier tests are also reported below.

Some of these genera have been reported as germinating easily while others have proved difficult, requiring cold, light, special soil or some other special condition in order to produce seedlings. The experiment reported below was planned to cover as many of these conditions for each species as the available seed supply permitted.

Tests designed to show whether the seeds were easy to germinate were made at constant temperatures of 1°, 5°, 10°, 15°, 20°, 25°, and 30° C., as well as several daily alternating temperatures. In each of the latter cases, the cultures were left at the higher temperature for eight hours and at the lower temperature for sixteen hours daily.

If the seeds did not germinate promptly under any of the conditions described above, low temperature pre-treatment was given at 1°, 5°, or 10°

C. for two weeks, and one, two, three, or four months followed by transfer to higher temperatures for germination. Light sensitivity was determined by tests in which the various seeds were placed on moist filter paper and kept in the dark, in daylight, or under red or blue light sources. The light source consisted of a pendent bulb covered by a metal shade which was closed at the top. The bottom of the shade was covered with cellophane of the color desired. A 60-watt frosted bulb was used in the lamp with blue cellophane, and a 50-watt bulb with the red, the bulb being at a distance of 12 inches from the top of the petri dish. The seeds which were to remain in darkness during the experiment were placed in petri dishes which had been painted on the outside with black Duco. These dishes were then placed in a petri dish can. The seeds were watered in darkness and germinations recorded at the end of the experiment. The experiments described above were performed using moist filter paper or moist granulated peat moss. The cultures were examined two or three times weekly and germination counts were made when the young radicle had started to grow.

Plantings were made using soil as a medium and in these cases appearance of the young shoot was taken as an index of germination. Soil plantings were made at various times throughout the year and the planted pots were placed outside to be wintered in open, board-covered, or mulched and board-covered cold frames. These plantings served the double purpose of determining the best time of year for planting and of testing the seed viability at intervals during the year. Two greenhouses with temperatures of approximately 21° and 13° C. were used to determine the seedling production from dry seeds. Pots of seeds were also placed in a 21° C. greenhouse after having been in cold rooms at 3°, 5°, or 10° C. for one, two, three, or four months.

For the plantings in the greenhouse and cold frames the results of seven workers (2, 3, 6, 7, 17, 19, 23) were studied and the following method used. In the case of larger seeds, broken or crushed gravel was placed in the bottom of the flat for drainage. The flat was then filled to within one inch of the top with a mixture of sand, humus, and sterilized sod soil in equal parts. The seeds were sprinkled on top, pressed in lightly and covered with a thin layer of soil.

The small seeds were planted in four and one-half inch pots made up in the same way. However, the soil was very finely sifted, and the seeds were covered to about the depth of the seed with sand which had been sieved through a number 40 mesh. This served to hold the seeds in place. The pots were protected with watch glasses which fitted the top of the pot perfectly and were packed in flats of moist granulated peat moss from which the soil received a more constant and uniform moisture supply. This eliminated the necessity of watering the soil directly and disturbing the small seeds. The pots were then placed in the greenhouses or in the cold frames.

TABLE I

THE EFFECT OF TEMPERATURE ON THE GERMINATION AND SEEDLING PRODUCTION OF ROCK GARDEN SEEDS. 100 SEEDS IN EACH TEST UNLESS OTHERWISE INDICATED. SEEDLINGS WERE PRODUCED IN THE FALL IN THE GREENHOUSE OR COLD FRAMES BEFORE COLD WEATHER UNLESS MARKED

Temperature °C.		Percentage germination or seedling production															
		*C. bar.**	*C. gar.*	*H. cor.*	*P. amb.*	*P. pul.*	*D. aiz.***	*G. lag.*	*M. lang.*	*P. den.*	*P. obc.*	*R. pyr.***	*C. mac.***	*C. leicht.***	*L. red.*	*D. alp.*	*M. cam.*
Constant	1	66	69	0	—	0	—	—	—	0	0	0	—	—	94	2	—
	5	93	40	24**	86**	21	87	60**	—	0	0	0	85	92	77	—	77
	10	94	49	59**	73**	16	96	34**	56	20	0	3	—	—	28	2	—
	15	92	81	92**	76**	35	—	48**	50	35	0	30	—	0	6**	0**	0
	20	91	74	—	68**	13	96	46**	30	38	0	65	—	—	—	2**	0
	25	73	0	—	10**	0	10	24**	18	48	1	72	—	0	6**	8**	0
	30	4	0	—	—	—	—	—	8	5	0	0	—	—	—	—	—
Alternating	1 to 20	—	—	—	—	40	—	—	—	—	—	—	—	—	—	—	—
	1 to 25	—	—	—	—	—	—	—	—	3	—	—	—	—	—	—	—
	5 to 20	83	82	—	92**	40	86	38**	—	38	—	0	—	—	—	0**	0
	5 to 25	93	73	—	72**	47	—	50**	76	28	—	1	—	0	16**	2**	0
	5 to 30	—	—	—	—	28	74	—	—	1	—	—	—	0	—	4**	0
	10 to 20	96	85	—	86**	37	—	42**	68	70	2	2	—	1	22**	4**	0
	10 to 25	98	76	96	76**	42	84	34**	70	64	1	3	—	1	—	2**	0
	10 to 30	95	67	—	86**	30	—	30**	62	79	0	1	—	0	—	6**	0
	15 to 30	92	51	94	76**	37	86	30**	56	71	3	1	—	0	16**	4**	0
	20 to 30	93	4	—	—	—	—	—	16	48	7	69	—	—	—	—	—
(Greenhouse)	13	51†	4	51	8	23	90	17	84	43	—	19	0	2	1	22	0
(Greenhouse)	21	72	73	74	25	15	88	37	87	72	36	68	0	0	2	3	0
Open frame		75	52	—	9	0	—	5††	—	0	—	0††	—	50‡	—	—	8‡
Mulched frame		—	—	—	4	0	—	4††	17††	31††	—	41††	80‡	100‡	63‡	52‡	89‡
Board-covered frame		85	70	75	10	0	94	19††	71††	50††	55††	30††	90‡	102‡	32‡	6‡	94‡

* *C. bar.* = *Campanula barbata*; *C. gar.* = *Campanula garganica*; *H. cor.* = *Hypericum coris*; *P. amb.* = *Pentstemon ambiguus*; *P. pul.* = *Primula pulverulenta*; *D. aiz.* = *Draba aizoides*; *G. lag.* = *Gentiana lagodechiana*; *M. lang.* = *Mimulus langsdorfii*; *P. den.* = *Primula denticulata*; *P. obc.* = *Primula obconica*; *R. pyr.* = *Ramondia pyrenaica*; *C. mac.* = *Calochortus macrocarpus*; *C. leicht.* = *Camassia leichtlinii*; *L. red.* = *Lewisia rediviva*; *D. alp.* = *Draba alpina*; *M. cam.* = *Meconopsis cambrica*.

** 50 seeds used.

† Record not complete—seedlings destroyed.

†† Seedlings produced in spring because the outside temperature was too cold for earlier germination.

‡ Seedlings produced in spring after exposure to cold temperature outside.

The open frame had no covering at all, and any pot placed in this frame had only the watch glass protecting it. The board-covered frame was well fitted with sashes over which slats were placed to prevent breakage of the glass. Here the snow and the strong winds could not get to the plantings except through cracks. The soil froze in these pots but they were not exposed to the sudden and extreme changes of temperature characteristic of the open frame. In the mulched frame, there was a deep layer of leaves under sashes and slats. These pots were frozen several times because the mulch was too light. The temperature of the greenhouses could be controlled only as long as the outside temperatures were lower than those inside. Thus the lower temperature greenhouse lost its value and had practically the same temperature as the higher temperature greenhouse by the end of April. During the summer the temperature of both greenhouses went up to as high as 35° or 40° C. in spite of the shading on them.

RESULTS AND DISCUSSION

The 17 species from which results were obtained have been grouped according to their type of response to the various tests. Those tests which did not add to the simplest method of obtaining good results have been omitted from the tables. For example, if low temperature pre-treatment did not aid germination, or if the light tests were unsuccessful, the data were not included.

Seeds Which Germinate Easily

The first group consists of those seeds which germinated easily over a rather wide range of temperatures. *Campanula barbata*, *C. garganica*, and *Hypericum coris* are in this class.

Arends (4) states that species of *Campanula* can be cultivated if they are a pure strain. According to Lüdi (18) *C. barbata* germinates only after cold treatment while McCully (19) writes that *Campanula* germinates easily when sown late in June.

Results obtained here also indicate that *Campanula* seeds are relatively easy to germinate. Tests of *C. barbata* made at controlled temperatures in 1931 gave 21 per cent germination at a daily alternating temperature of 15° to 30° C. In 1936, seeds sown in the 21° C. greenhouse produced 16 per cent of seedlings within three weeks after planting. When they were given one month of low temperature pre-treatment at 5° C., 28 per cent of the seedlings appeared one week after the pot was transferred to the 21° C. greenhouse. A complete test was made on this species beginning October 15, 1937. The seeds germinated very readily in the ovens at all temperatures except 30° C. within two weeks after planting (Table I). The response at the lower temperatures was not quite as prompt even though the final percentages were as high as those of the seeds at the higher temperatures. This was also true of the seeds planted outside in the cold frames and in the 13° and 21° C. greenhouses (Table I). The results of the plantings in the mulched and board-covered frames were averaged as the seedlings were produced in the fall before the mulch was laid. Table II shows the results of planting these seeds at intervals during the year. During the coldest months the seeds were planted in the 21° C. greenhouse and after February in both the greenhouse and the board-covered frame. The spring and September plantings were better in the board-covered frame than in the greenhouse. The planting made in September, 1938 gave 65 per cent seedling production in the board-covered frame outside indicating very little or no loss in vitality with 11 months of storage.

C. garganica seeds also germinated easily, but the range of favorable temperatures was not as wide as for *C. barbata* (Table I). In an earlier experiment the 21° C. greenhouse had been used but only 8 per cent of the seedlings appeared. In most cases a very good stand of seedlings was obtained in the current experiment in the 21° C. greenhouse within one month after planting (Table I). However, replicate tests gave a wide variation in seedling production, which could be attributed to the difficulty in establishment of the seedlings in the soil after germination. Although precautions were taken to prevent seedling loss, there was a great variation in number from day to day. These seedlings were among those most susceptible to damping off. Such irregularities were probably the cause of the poor seedling production in the 13° C. greenhouse since this temperature was favorable for germination (cf. oven tests). The protection afforded by the sashes and slats outside resulted in a good stand of seedlings before the cold weather set in (Table I). Plantings in the 21° C. greenhouse during the winter and in the board-covered frames during the spring gave good results (Table II). This species also failed to show a loss in vitality with 11 months of storage.

Limited tests on fresh seeds of *Hypericum coris* showed that they respond in the same manner (Table I) and with about the same speed as *Campanula* species. The greenhouse planting in September showed that these seeds had not lost their vitality with storage (Table II).

None of these three species was light-sensitive, and pre-treatment at 1°, 5°, or 10° C. did not improve either the percentage germination or seedling production.

Pentstemon ambiguus and *Primula pulverulenta* also germinated very readily but seedling production in the greenhouses was erratic or poor.

In 1936, 12 different species of *Pentstemon*, many having seeds of different ages, were tested for viability. They were placed on filter paper in petri dishes at 10° to 20° C. daily alternating temperatures and at the same temperature after different periods at 5° C. Results are shown in

TABLE II

SEEDLING PRODUCTION FROM PLANTINGS MADE AT VARIOUS TIMES DURING THE YEAR. 100 SEEDS IN EACH LOT UNLESS OTHERWISE INDICATED

Date of planting	Percentage seedling production													
	*C. bar.**		*C. gar.*		*H. cor.*		*P. amb.*		*D. aiz.*		*G. lag.*		*M. lang.*	
	GH**	BC	GH	BC	GH	BC	GH	BC	GH	BC	GH	BC	GH	BC
1937														
Oct.	72	85	73	70	74	75	—	—	88	94	—	—	—	—
Nov.	—	—	—	—	—	—	—	—	—	38	—	—	—	—
Dec.	69	—	78	—	53	—	25	10	86	—	—	—	87	11
1938														
Jan.	50	—	61	—	—	—	—	—	—	—	37	19	87	35
Feb.	58	66	65	70	31	38	41	11	—	44	32	35	82	53
Mar.	38	80	9	73	—	—	0†	—	—	—	—	62	54	—
Apr.	30	81	3	84	20†	58†	—	2†	—	—	28†	0	40†	86†
May	—	—	—	—	—	—	—	—	—	68	32†	0	—	—
June	—	—	—	—	—	—	—	—	—	—	26†	0	58	73
July	0	13	54	37	—	44	38†	—	—	48	—	—	—	—
Aug.	10	—	—	—	—	—	—	—	—	—	—	—	—	—
Sept.	7	65	—	67	70†	—	—	—	—	—	—	—	86†	—

	P. den.		*P. obc.*		*R. pyr.*		*C. mac.*	*C. leicht.*	*L. red.*	*D. alp.*	*M. cam.*
	GH	BC	GH	BC	GH	BC	BC	BC†	BC	BC	BC
1937											
Oct.	—	—	—	—	—	—	90	—	32	10	94
Nov.	71	50	—	—	—	—	—	100	—	40	84
Dec.	84	—	—	—	69	30	—	94	79	57	38
1938											
Jan.	53	—	—	—	64	40	70†	24	43	32	2
Feb.	71	30	—	—	63	27	—	80	37	4	2
Mar.	83	48	—	—	75	—	—	—	—	—	0
Apr.	71	99	—	—	—	—	—	88††	—	—	—
May	—	—	36	55	9	42	—	—	72†, ††	38†, ††	—
June	—	71	33	51	—	—	—	—	—	—	—
July	—	—	23	50	63	43	—	—	—	—	—
Aug.	—	39	22	19	—	—	—	—	—	—	—
Sept.	33	45	—	—	39	4	—	—	—	—	—

* See footnote* Table I for key to identification of the species.

** GH = greenhouse at 21° C.; BC = board-covered cold frame.

† 50 seeds used.

†† Placed in 5° C. room until seedling production was well advanced, then transferred to greenhouse.

Table III. *P. hirsutus* (L.) Willd. and *P. spectabilis* Thurb. germinated only after low temperature pre-treatment. *P. ambiguus* was improved by low temperature pre-treatment, while all the others germinated at 10° to 20° C. directly with varying degrees of success. In several cases the older seeds germinated better than the fresh seeds.

The 1937 lot of *P. ambiguus* gave excellent germination at 5°, 10°, 15°, 20°, and at daily alternating temperatures of 5° to 20° C., 5° to 25° C., 10° to 20° C., 10° to 25° C., 10° to 30° C., and 15° to 30° C. (Table I). Although a constant temperature as high as 25° C. inhibited germination, these seeds tolerated a temperature of 30° C. when alternated with 15° C. or lower. Germination began within three days. Very few of the seedlings planted in the greenhouse from oven germinations became established. Seedling production in the cold frames and greenhouses was very poor (Table I) and low temperature pre-treatment at 5° C. did not help to overcome this condition. The seedlings in the greenhouse tended to become a translucent green, curl up, and die, nor were plantings at monthly intervals very successful. Best results were obtained from the planting in the 21° C. greenhouse in February but the counts on this lot are so irregular, due to difficulty in seedling establishment, that the total seedling production given may not be accurate. More experiments will be conducted upon this species as soon as fresh seeds are obtained in order to determine the cause of this.

TABLE III

RESULTS OF PRELIMINARY TESTS MADE ON PENTSTEMON SPECIES OF DIFFERENT AGES IN 1936

Species	Age of seed, years	Percentage germination at				
		5° C.	10° to 20° C. after weeks at 5° C.			
			0	2	4	7
P. ambiguus Torr.	3	—	55	58	86	81
P. barbatus Nutt.	8	—	83	87	56	53
P. centranthifolius Benth.	1	38	48	*	—	—
P. cobaea Nutt.	3	—	80	77	—	—
P. cobaea	1	75	84	78	—	—
P. cobaea	Fresh	3	23	22	16	—
P. connatifolius	11	1	3	0	1	—
P. connatifolius	7	—	62	43	56	*
P. connatifolius	Fresh	36	44	40	35	—
P. eatonii Gray	6	38	43	10	*	—
P. eatonii	1	78	76	75	—	—
P. eatonii	Fresh	79	81	76	—	—
P. hirsutus Willd.	1	—	1	79	92	89
P. microphyllus Gray	1	45	57	1	20	—
P. palmeri Gray	Old	47	69	25	25	—
P. palmeri	6	—	25	15	3	0
P. palmeri	1	60	54	28	46	—
P. palmeri	Fresh	—	17	13	8	*
P. parryi Gray	9	—	7	1	2	0
P. parryi	2	*	5	3	3	0
P. parryi	Fresh	2	2	2	5	2
P. secundiflorus Benth.	2	25	47	41	37	*
P. spectabilis Thurb.	1	—	7	42	87	96

* Seeds had started to germinate.

The finding of Snowberger (28) that species of *Pentstemon* require much freezing for germination was not corroborated by the work done here. Rather they were found to require a relatively low temperature (not higher than 20° C.) for germination. This would explain failure of these seeds to germinate if planted late in spring.

In a test made in 1931, *Primula pulverulenta* gave 21 per cent germination at 15° to 30° C. daily alternation and none in the 21° C. greenhouse. The highest germination obtained in the 1937 test was 40 to 47 per cent at daily alternating temperatures of 1° to 20° C., 5° to 20° C., 5° to 25° C., and 10° to 25° C. (Table I). Germination began after three weeks and continued for nine more weeks. Again seedling production in the greenhouse was inferior to the germination in the ovens. Many of the seedlings in the ovens looked abnormal as did those in the greenhouse. Further work is necessary to determine whether this difficulty was caused by a poor seed lot, wrong medium, or other unfavorable conditions. The few seedlings which did survive were strong.

Seeds Affected by Light

The second group of seeds was not dormant but exhibited a response to light. *Draba aizoides*, *Gentiana lagodechiana*, *Mimulus langsdorfii*, and *Primula denticulata* did not appear to require light for germination but exposure to light permitted germination at temperatures ordinarily prohibitive. *Primula obconica* and *Ramondia pyrenaica*, on the other hand, required light for germination.

A 1936 test on seeds of *Draba aizoides* gave negative results. There were not enough seeds in the current lot for complete tests, but the results are quite definite. All the oven temperatures tested except 25° C. proved to be very good for seed germination, which was complete in one month (Table I). Seeds planted in the fall gave an excellent stand of seedlings before winter (Table I). After February, the vitality tests were made in the board-covered frame so that the test could also serve the purpose of determining the best time for outside planting (Table II). The seeds had not lost their vitality by the following May (Table II) and the drop in seedling production in July was probably due to the high temperature, but as there were no more seeds, further tests could not be made. When the seeds were placed under lights covered with red or blue cellophane at about 25° C. high percentages in germination, 80 and 70 per cent, were obtained (Table IV). This compares very favorably with the 96 per cent obtained at 20° C. without continuous light (Table I). Only 6 per cent of the seeds kept in darkness at the same temperature germinated. It should be mentioned that seeds from all controlled temperatures received some light as the cultures were removed from the ovens and examined in light at room temperature. The dark controls, on the other hand, were never exposed to light until the end of the experiment. In the greenhouse plantings, the sand covering was not deep enough to prevent the light from reaching the seed. This is true of all the light-sensitive seeds of this experiment.

There have been numerous reports on the germination of *Gentiana* species. Wocke (30) writes that gentians are found on sunny, stony slopes in good humus-rich garden soil, and do not require much attention. Giersbach (9) found that *G. acaulis* and *G. crinita* required low temperature pre-treatment while *G. andrewsii* required both light and cold temperature. Musgrave (23) reports that moist soil with good drainage is best and that gentians germinate better in the cold frame than in heat. With this treatment they require several months more but are much stronger. According to Lüdi (18) *Gentiana* germinates only after the second year. Amsler and Lawrence (1) sowed the seeds in the fall, scarcely covering them, and exposed the pans to one or two frosts. Britton (5) planted the seeds of fringed gentian immediately after harvest in the fall in a moist shady place. Norton (25) collected the seeds after the first frost, stored them in damp sand over winter, and sowed them early the following spring. Hedden (14) froze the seeds and let them thaw slowly in a cool place. It is apparent from the evidence here reported that the different species of *Gentiana* vary greatly in their germination requirements. Some require light or low temperature while others germinate without any special treatment.

In the controlled temperature tests of *G. lagodechiana*, approximately the same germination percentages were obtained over a wide range of temperatures with some decrease for the higher temperatures (Table I). Germination was complete at the higher temperatures within six weeks, but at 5° C. three months were necessary to obtain the total of 60 per cent. Three months' pre-treatment at 1° C. increased the germination at 20° C. from 46 to 70 per cent. Light tests at 20° to 22° C. showed 66 per cent germination under the red, and 54 per cent under the blue (Table IV) with slightly better root development in the latter case. No seedlings were produced in the dark control.

TABLE IV

RESPONSE OF CERTAIN ROCK GARDEN SPECIES TO DIFFERENT LIGHT CONDITIONS; 100 SEEDS EACH LOT

Light condition	Percentage germination							
	*D. aiz.**	*G. lag.*	*M. lang.*	*P. den.*	*P. obc.*		*R. pyr.*	
Red	80	66	20	89	57	90	96	95
Blue	70	54	10	4	23	18	90	91
Dark	6	0	0	0	0	0	0	0
Room temperature Light	—	—	—	—	—	80	88	94
Room temperature Dark	—	—	—	—	—	0	0	0

* See footnote* Table I for key to identification of the species.

Seedling production in the soil (Table I) was inferior to germination on filter paper as the seedlings damped off quite readily. Because of this erratic behavior it was very difficult to obtain accurate greenhouse and cold frame counts. It was also difficult to judge whether the seeds had lost any of their vitality. The only good seedling production occurred in March in the board-covered frame. As soon as more seeds are obtained, experiments on this species will be continued.

Results of tests on *Mimulus langsdorfii* in the greenhouse were slightly better than those in the ovens (Table I). The highest percentages in the greenhouses were 84 and 87 per cent in three weeks as compared with 76 per cent in the ovens in two weeks. Two tests made under the lamps with cellophane and in darkness (Table IV) at a temperature of about 25°C. showed *M. langsdorfii* germination to be slightly better in the light. In this case, however, light was less effective in bringing about germination at an unfavorable temperature than in *Draba aizoides*. The superiority of the greenhouse plantings to those in the ovens may have been due to the extra light received by the former. It is possible that optimum germination conditions for *Mimulus* should combine light with a favorable temperature. Additional tests are needed to establish this point. Seedling production from monthly tests of *M. langsdorfii* in the greenhouse was somewhat reduced in March, April, and June plantings due to higher temperatures (Table II). These seeds retained complete vitality after 11 months of storage.

Our results are in agreement with Mitchell (22) and Hutchings (16) who state that *Mimulus ringens* is light-sensitive. Response to exposure to light in this case was found to be roughly proportional to the intensity with no germination at less than 1.5 foot candles. Furthermore, a daily light period of two hours gives slow and incomplete germination which becomes more rapid and more complete with increased exposure. The latter also mentions an article by Diller in which he found *Mimulus* seeds to be light-sensitive.

Several reports have been published on the germination of *Primula* seeds. According to McKenzie (20) *P. denticulata* and *P. pulverulenta* can be raised from seeds sown in June or July and placed in shaded frames. Worth (31) says to sow on top of a fine well-drained soil about June 1, put into a cold shed, and cover with black building paper until germination begins, after which the boxes should be placed in shaded frames. Herele (15) states that light is unfavorable to *P. obconica* while Lüdi (18) says that frost is necessary for the germination of *Primula*. Grullemans (11) finds that much lime, preferably in the form of old mortar mixed with an equal amount of soil, is required. McKenzie (20) writes that although the seeds of all primulaceous plants are rather erratic in germination, they cannot be termed difficult, except some of the alpine species. Arends (4) recommends that many of the primulas be planted in boxes and pots and labeled "difficult to germinate."

In the present tests best germination of *P. denticulata* occurred at daily alternating temperatures where 10° or 15° C. was used as the lower and 20°, 25°, or 30° C. as the higher temperature (Table I). Up to 72 per cent seedling production was obtained within three to five weeks in the 21° C. greenhouse, whereas the 13° C. greenhouse or a winter outside was less effective. It will be noted (Table IV) that blue light inhibited the germination of *P. denticulata* seeds while red light permitted germination up to 89 per cent. This same effect, though not so marked, was evident in studies with seeds of *P. obconica* reported below. The vitality tests show a drop in August and September (Table II). Further tests to determine whether this was a temperature effect were not possible as the seed supply was exhausted.

The lot of seeds of *P. obconica* received in the summer of 1937 molded very badly and germinated very little and in a very erratic manner. Since it seemed likely that these seeds were not viable the authors obtained 100 plants which set many seeds as a result of hand pollinations. The ripe seeds were collected and germination tests begun immediately. Seeds collected May 4, 1938 were tested May 7. The highest percentage obtained in the ovens was 7 per cent at a daily alternation of 20° to 30° C. Under red light and in the frames outside, however, the germination and seedling production were 57 and 55 per cent respectively within six weeks. Seeds collected the end of May, on which tests were begun June 16, gave 79 per cent seedling production in the greenhouse. The same seeds tested one month later produced equally good results. The earlier collection of seeds has never improved beyond the original 57 per cent, whereas the different lots collected after about May 10 have given consistently better results. This may mean that the earlier lot of seeds was collected either before the seeds were mature or before the plant was producing its best crop. Seeds collected later in the summer have been tested constantly giving as high as 90 per cent in the greenhouse. Since seeds of *P. obconica* were shown to be light-sensitive (Table IV), a further test was made to determine whether cold temperature could take the place of light. The results showed that the light requirement of *P. obconica* could not be replaced by cold temperature for the low temperature did not increase germination in the light nor did it cause germination in the dark. Storage tests on these species indicated that if the seeds were thoroughly air-dried when stored they still retained their vitality in open and sealed vials at 5° C. and at room temperature after five months. Those which were not thoroughly dried when stored had their vitality considerably reduced in the 5° C. storage and completely at room temperature in five months. Many of the reported failures to germinate these seeds can doubtless be attributed to their short life span under ordinary storage conditions.

The seeds of *Ramondia pyrenaica* responded very definitely to rather high temperatures, 20° C. (65 per cent), 25° C. (72 per cent), and 20° to 30° C. daily alternation (69 per cent), as shown in Table I. Germination was inhibited, however, at 30° C. Table IV shows that there was considerable improvement after constant exposure to light (96 per cent) at 20° C. The 21° C. greenhouse planting had a good percentage seedling production (68 per cent) whereas the plantings in the 13° C. greenhouse and in the cold frames yielded considerably less (0 to 41 per cent). Plantings in the board-covered frame at intervals during the year proved inferior for seedling production to those of the 21° C. greenhouse, except for the May planting. The cause for the drop in seedling production from seeds planted in May in the 21° C. greenhouse we do not know unless perhaps the moisture balance was upset or some other external factor influenced it. It is certain that it was not due to loss of vitality for the July planting gave very good results. When the seedlings appeared above ground after three weeks to two months they were very tiny, and succeeding counts two days apart often showed a drop of 20 to 30 per cent in number without any apparent cause for the decrease. Sometimes an equal increase was observed two days later. Because of this peculiarity it was very difficult to obtain an accurate total seedling production in the greenhouse or cold frames. However, those seedlings that remained, although tiny, seemed to be sturdy and grew very slowly but steadily.

Further studies were made to determine the degree of light sensitivity of these seeds. Duplicate lots of 100 seeds each were placed under red and blue light and in darkness at 15° and 25° C., and in light and dark petri dishes at room temperature, and in the 21° C. greenhouse. The seeds exposed to light under the different conditions germinated up to 95 per cent. Those kept dark under the same conditions gave no seedlings. The seeds which had been on moist filter paper in darkness at room temperature for two months were transferred to the light at room temperature where 83 per cent germinated. This showed that light was essential for germination but that the seeds were not injured when kept in a dark germinator for two months. Another series of tests was then begun to determine whether low temperature pre-treatment could take the place of light. Seeds were placed in 1°, 5°, and 10° C. for one and two months. Some lots were then transferred to the 20° and 25° C. ovens, where they would receive light when watered twice weekly. Others were transferred to a 15° C. or 25° C. room and placed under a red or blue light, or in darkness. Seeds exposed to light after low temperature germinated, those kept in darkness did not, whereas those placed in the ovens gave only a few germinations. Thus low temperature pre-treatment did not take the place of light in the germination of *Ramondia pyrenaica* seeds.

Seeds with Hard Coats

Cytisus decumbens was the only species studied which had a hard coat. In an earlier test, seeds of *C. praecox* Bean, a related species, were filed and planted in a 21° C. greenhouse, producing 43 per cent of seedlings. *C. decumbens* when filed and planted in a 21° C. greenhouse did not produce seedlings. The supply of seeds for the current experiment was not very large and only 25 seeds could be used for each test. Lots of seeds were treated with concentrated sulphuric acid for 15, 30, and 45 minutes. One lot of each treatment together with an untreated control was placed in moist granulated peat moss at 10° to 25° C. daily alternation. Samples of the same series were planted in soil and placed in the 7° C., 13° C., and 21° C. greenhouses and in the 5° C. room. At 10° to 25° C. daily alternation 12 per cent of the untreated seeds germinated, while 67 to 90 per cent of the seeds treated with sulphuric acid germinated. In the greenhouse plantings also the treated seeds gave a better stand of seedlings than the untreated. The length of treatment necessary to obtain optimum results varied at the different temperatures and more extensive experiments should be performed to obtain complete data.

Seeds Requiring Low Temperature for Germination

Calochortus macrocarpus, *Camassia leichtlinii*, and *Lewisia rediviva* belong to this group. These seeds are not dormant, but require low temperature for germination.

Eighty-five per cent of the seeds of *Calochortus macrocarpus* germinated within two months at 5° C. (Table I) while sample lots removed from 5° C. and planted in the 21° C. greenhouse gave only 6 per cent seedling production. When the seedlings which germinated at 5° C. were planted in the 21° C. greenhouse they appeared above ground within a few days and grew well. Seeds planted in the mulched and board-covered frames in October, 1937 and in the board-covered frame in January, 1938 gave very good stands of seedlings in the spring of 1938 (Tables I and II). The seedlings have since died back and bulblets have been formed. A previous experiment gave no seedlings because the seeds were not left long enough at 5° C. These results agree with those of Purdy (27) who planted *Calochortus* seeds in January and had seedlings March 1. There seems to be no doubt that these seeds require cold for germination. Williamson (29) planted the seeds in open sandy soil with leaf mold and left them for a full year with good results.

In a former experiment, *Camassia leichtlinii* seeds gave 30 per cent seedling production in the 21° C. greenhouse after one month pre-treatment at 5° C. The present lot of seeds did not respond to that treatment. They did not germinate at high temperature directly or at high temperature after pre-treatment at low. However, 92 per cent (Table I) of the seeds germinated at 5° C., and when seed lots which had been at high temperatures for several months were transferred to 5° C. they germinated very well. Excellent seedling production occurred in the cold frames but not in the warm (21° C.) or cool (13° C.) greenhouses (Table I). The January planting was lost because the soil in the pots became too dry. It was almost impossible to test the vitality of *Camassia* seeds in the greenhouse during the summer because of the warm temperature. In the most recent vitality tests, however, the pots were placed in a 5° C. cold room for two or more months. They were watched carefully and removed to a light greenhouse as soon as the seedlings appeared. In spite of every precaution in shading them, they did not always survive when transferred to the higher temperature of the greenhouse. At first, the *Camassia* seeds were planted to a depth equal to the size of the seed. However, this was too shallow as part of the hypocotyl appeared above the soil in germination. The seeds are now planted at a depth of three-quarters of an inch.

Lewisia rediviva was not quite as definite in its low temperature requirement as *Calochortus* and *Camassia* (Table I). It germinated to a small degree in some of the alternating temperatures but a low constant temperature gave the best results. Those lots which had been at high temperatures and had apparently ceased germination gave many additional seedlings when transferred to 1° C. Cold frame plantings were better than greenhouse plantings in this species also, as even the 13° C. greenhouse was not cold enough to be effective. Vitality tests on this species are being conducted in the same manner as those of *Camassia*, but results are incomplete. Seedlings of *L. rediviva* did appear at a high temperature if the seeds were about to germinate when placed at the high temperature. *Camassia* and *Calochortus* did not give this response. Earlier experiments on *L. rediviva* were unsuccessful because the proper temperature had not been used.

Dormant Seeds

The last group of seeds consisted of those species the seeds of which were dormant and required low temperature pre-treatment to germinate. *Draba alpina* and *Meconopsis cambrica* were in this group.

In previous tests, *Draba alpina* produced no seedlings in the greenhouse or after one month of exposure to 5° C. before transfer to the greenhouse. Unlike *D. aizoides*, *D. alpina* in the present tests germinated very poorly, up to 8 per cent, at the various constant and alternating temperatures (Table I). When germination had ceased at these temperatures the seed lots were all transferred to 1° C. for one month, after which they were all placed at 10° to 30° C. daily alternating temperature where the germination percentage soon reached 56 per cent. However, the high temperature preceding the low was not necessary, for two months' exposure to 10° C.

before transfer to 10° to 30° C. resulted in 55 per cent germination. A different combination of high and low temperatures might have given better results. Plantings in a mulched cold frame over the winter (Table I) were more successful than those in the greenhouses over the same period. However, one month's exposure to the high temperature of the 21° C. greenhouse before placing in the board-covered frame for the rest of the winter resulted in 71 per cent seedling production (Table II). Pre-treatment in the 5° C. room for three months followed by transfer to the 13° C. greenhouse gave 56 per cent seedling production. Vitality tests are not yet complete. In December the viability was still good and the January planting showed promise of good results until it was lost by drying. Later plantings may not have had enough cold weather to produce a good stand of seedlings. As soon as fresh seeds are obtained, further tests on this species will be made.

Meconopsis sown in February on the surface of the soil and placed in a moist growing temperature germinated well according to Harrow (13). Harley (12) recommended a good moisture supply during the growing period but added that if they did not grow well, the plantings should be left until the following spring. When *Meconopsis* was watered with a fine spray and left to freeze, Archie (3) found that germination occurred 10 days after removal to a cold frame or any warm place.

Experiments on *Meconopsis cambrica* performed here showed that plantings made and left in the cold frames over the winter gave excellent seedling production (Table II) whereas greenhouse plantings were totally ineffective. Seed lots left in the 21° C. greenhouse for one or two months before transfer to the cold frames also germinated well. It is obvious that these seeds are dormant requiring a period of low temperature before they will germinate to any extent. There was a drop in seedling production in those pots which had remained in the 21° C. greenhouse until February before transfer to the cold frame. Probably there was not enough cold temperature after February to after-ripen the seeds. The vitality tests (Table II) gave increasingly poor results after December, caused probably by too little exposure to cold without previous high temperature. It was noted that the best results occurred when the seedling production began before the middle of April especially if the seeds had not been exposed to much cold. The earlier the germination began the better were the results. The summer plantings were poor in spite of low temperature pre-treatment probably because the greenhouse temperatures were too high for actual germination.

These results were borne out by tests at controlled temperatures. Seeds placed in the various high temperature ovens did not germinate. Therefore, those seeds in the ovens at a temperature above 15° C. were transferred to

5° C. for two months and then placed at 5° to 20° C. daily alternating temperature where germination occurred. The seeds which had been held at 25° C. and 20° to 30° C. before transfer to low temperature gave the highest germination (78 and 82 per cent).

Further experiments were performed to determine whether it was necessary to have high temperature preceding the low temperature as suggested by the experiment above. Seeds treated for two months at 5° C. and then transferred to 5° to 20° C. germinated up to 67 per cent. Before the three-month period was up, the seeds at 5° C. were germinating and gave 77 per cent at that temperature. Seeds placed at 20° to 30° C. daily alternation for one month and then given two months' exposure to 5° C. germinated 85 per cent at 5° to 20° C. daily alternation. Germination occurred at a higher temperature than this after pre-treatment at low temperature only if the seeds were about ready to germinate. The results were better and more consistent when a lower temperature was used. A similar test was made using greenhouses instead of ovens but no results were obtained until the cooler temperatures of fall lowered the greenhouse temperatures. A 5° C. greenhouse is now being used with excellent results sufficing for both after-ripening and germination. The most practical method of handling these seeds is an October or November planting in a mulched or board-covered frame.

From the literature cited and the report given, it may readily be seen that although plants may be classified as rock garden or alpine plants, their treatment cannot be similarly unified. This variation is the fundamental basis of the difficulties encountered by growers. The genera vary greatly in their requirements for germination as do also the different species of the same genus.

Much of the difficulty comes from having empty or non-viable seeds. Some seed lots were found to be mixtures of two or more species and thus have given germination results which were not dependable. Data obtained from tests of these unsatisfactory seed lots have not been included in this report. Many of the seeds are very tiny and unless very great care is taken they are easily lost. Individual seed lots of the same species respond differently to the same treatment. This may be due to the difference in age of the plants from which the seeds are collected or to the maturity of the seed at the time of collection. Rock garden seeds are very sensitive to moisture also. They usually require plenty of moisture but good drainage must be provided. Sudden changes in atmospheric conditions easily upset this balance and since the seeds are planted very close to the surface of the soil the roots are readily affected. Richness and texture as well as acidity of the soil are factors in seedling production. Some seeds are dormant, requiring low temperature pre-treatment, others germinate only at low temperature, while still another group germinates readily over a wide temperature range. Other factors influencing germination are light sensitivity and hardness of seed coats.

Growth of the Plants Beyond the Seedling Stage

The method used for planting the seeds has been described previously. The seedlings were left in the pots until they were large enough to move. To eliminate as much injury as possible from frequent transplantings, the tiny seedlings were potted up immediately in two and one-quarter inch pots in a mixture of equal parts of sand, sterilized leaf mold, and sterilized sod soil. When repottings to three and one-half inch pots or larger were made, a mixture of cow manure, leaf mold, and sterilized soil in equal parts was used.

Campanula barbata and *C. garganica* were first planted October 15, 1937, and germinated quite promptly. The seedlings in the 21° C. greenhouse were potted up and left there for almost two months. Then half of the plants were transferred to a 13° C. greenhouse to see what effect the cooler temperature would have on the plants. They grew more slowly but were fuller and more compact in the 13° C. greenhouse whereas the plants in the 21° C. greenhouse were taller and more spindly. About the middle of April, plants of both species were taken from each greenhouse to the board-covered frame. *C. barbata* bloomed outside and in the greenhouses by the beginning of June and continued to bloom all summer. One seedling of *C. garganica* in the cold frames outside bloomed about the middle of July. The plants of these two species are being kept over the winter in various conditions to determine their hardiness.

Hypericum coris was found to be a very slow-growing plant. In warm temperatures it grew faster and more fully than in cool temperatures. Some of the plants were lost during the summer but those that survived the summer heat were strong.

Meconopsis cambrica grew well in both the 13° and 21° C. greenhouses. The plants bloomed earlier and well in the latter greenhouse but were taller and not quite as sturdy as those in the 13° C. greenhouse. As the weather outside became warmer, the plants in the cooler greenhouse in which the temperature could no longer be controlled became as large as those in the warmer greenhouse but remained fuller and produced more flowers. Plants placed outside also bloomed and seeds were collected from all three lots.

Mimulus langsdorfii seeds were planted December 28 in a warm greenhouse and 100 days later, seeds were obtained from the plants. *Mimulus* sent out many shoots which rooted immediately when placed in contact with water. Growth was less rapid at cooler temperatures. These plants grow more than one foot high and should not be placed among smaller plants.

Some *Pentstemon ambiguus* seedlings potted up about two months after planting when about one-half inch tall survived much better in the 21° C. greenhouse (2 per cent died) than in the 13° C. (50 per cent died) or 8° C. (33 per cent died) greenhouses. A later observation showed that the plants in the first set continued to grow, in the second they remained stationary, while in the last set more plants died. A later potting when the plants were ten weeks old was accomplished without loss of seedlings at 21° C. They are now about 4 inches tall and strong.

Draba aizoides and *D. alpina* seedlings after their first repotting were placed in 21° and 13° C. greenhouses. Those plants placed in the cooler greenhouse grew very full and well. One *D. alpina* plant had a few blooms in the cool greenhouse by the middle of March and *D. aizoides* was about to bloom when a warm period stopped it. Another cold period aided further bud formation and by the middle of April three plants of *D. aizoides* had bloomed. All the plants in the 21° C. greenhouse were taken to the cold frame at that time but it was too warm for bud development. In July an extended period of hot and rainy weather killed many of the plants.

Cytisus decumbens plants grew well in the 21° C. and in the 13° C. greenhouses. The 8° C. greenhouse was evidently too cold, for the seedlings were not as large and healthy looking there. During the summer they were all placed in the frames outside and were all strong and healthy in November.

Those *Gentiana lagodechiana* seedlings which did not damp off during the germination tests were potted up and placed in the 21° C. and 13° C. greenhouses. During the summer some of the seedlings were taken to the cold frame. All of the seedlings are in good condition.

Primula denticulata plants grew easily. However, cold temperature retarded their growth. All of the plants were taken outside the middle of May and grew very well there. One plant bloomed in June but the flowers were small and the head incomplete.

Primula obconica although not a hardy primrose is not difficult to grow. Seeds sown in a warm greenhouse on November 9 produced plants which bloomed the following June.

These experiments were begun between October 15 and December 28, 1937. When the seedlings were repotted, they were placed in greenhouses. In this way, early bloom was brought about. Seedlings from those seeds planted in the cold frames will not bloom until next year. This is also true for those plantings made during the spring and summer.

Calochortus macrocarpus, *Camassia leichtlinii*, and *Lewisia rediviva* will not be ready to bloom for several years.

SUMMARY

Campanula barbata, *C. garganica*, *Hypericum coris*, *Pentstemon ambiguus*, and *Primula pulverulenta* germinated well over a wide range of controlled temperatures. Greenhouse and cold frame tests were very successful for the *Campanula* species and *Hypericum*, but *Pentstemon ambiguus* and *Primula pulverulenta* were very erratic in their response. With the possible exception of *Hypericum coris* the seeds were still viable after eleven months' storage at room temperature.

Draba aizoides, *Gentiana lagodechiana*, *Mimulus langsdorfii*, and *Primula denticulata* also germinated well over a fairly wide temperature range. At temperatures as high as 25° or 30° C., however, fewer seedlings were produced. Tests in light and dark at these unfavorable temperatures showed that the presence of light induced germination. This points to the need of some pre-treatment for germination at certain temperatures. Low temperature pre-treatment was used instead of light to bring about germination of *Gentiana lagodechiana* seeds at unfavorable temperatures.

No germination of *Primula obconica* and *Ramondia pyrenaica* occurred even at favorable temperatures without light. However, the usual method of greenhouse planting where the seeds were on the surface of the soil and exposed to light produced seedlings readily.

Primula obconica seeds lose their vitality readily under ordinary conditions of storage unless precaution is taken for thorough drying.

Calochortus macrocarpus, *Camassia leichtlinii*, and *Lewisia rediviva* produced seedlings at 5° C. within two to three months after planting. This low temperature was necessary for germination and not for breaking dormancy. Fall or winter plantings should therefore be made two or three months before the arrival of warm weather. Although both mulched and board-covered cold frames furnished favorable conditions for germination the latter is to be preferred in the region of Yonkers, New York, since the seedlings are likely to appear before the removal of the mulch and be lost.

When *Cytisus decumbens* was treated with concentrated sulphuric acid for from 15 to 45 minutes, a good percentage of seedlings was produced.

Draba alpina and *Meconopsis cambrica* produced good stands of seedlings in the spring if planted in the fall and kept in cold frames over winter.

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FERTILIZER VALUE OF COLLOIDAL PHOSPHATE

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The need for additional facts with respect to the physico-chemical properties and soil improving value of colloidal or Florida pond phosphate and requests for its investigation were brought to our attention recently. The requests were made because certain state chemists and agronomists considered the claims with respect to its availability to plants and its soil improving value by sellers of it to be exaggerated. The colloidal or pond phosphate is present in the matrix of the phosphate deposits. As the matrix is washed in preparation for market, the colloidal suspension is collected in reservoirs or ponds. The water disappears and the suspended materials remain. Owing to these requests and the fact that there are several million tons in the various deposits, laboratory studies and greenhouse and field tests with respect to its fertilizer value have been conducted.

LITERATURE REVIEW

Hill *et al.* (7), and Jacob *et al.* (8) found the iodine content of colloidal phosphate to range from 50.8 to 63.5 parts per million, VaO 0.03, CuO less than 0.0005, Ba_2O_2 less than 0.001, and ZnO 0.005 per cent. The Tennessee brown rock phosphate carried from 9.8 to 28.2 parts per million of iodine in addition, Ba_2O_2 0.005 to 0.007, CuO 0.002 to 0.003, and ZnO 0.001 to 0.005 per cent.

Fraps (4) maintains there is no trustworthy chemical method by means of which the availability to plants of the P_2O_5 in colloidal and rock phosphate can be determined. The ammonium citrate and citric acid extraction method, however, has been employed in studying their properties. Jacob *et al.* (8) for example found the average amount of ammonium citrate soluble P_2O_5 in nine samples of colloidal phosphate to be 0.58 and the average amount of citric acid soluble P_2O_5 6.53 per cent. The average amount of citrate soluble P_2O_5 in six samples of Tennessee brown rock phosphate was 0.94 and that of citric acid soluble P_2O_5 in them was 5.88 per cent. Haskins (5) reports a sample of colloidal phosphate taken in 1930 to contain 0.21 per cent citrate soluble P_2O_5 and another one collected in 1937 to carry 2.88 per cent (6).

The difference in the ability of various crop plants to utilize the phosphorus of raw phosphates is an important agronomic consideration in the use of raw phosphates for soil improvement. By means of pot cultures Bauer (2) determined the effectiveness with which various plants can make use of raw phosphate on one hand and superphosphate on the other as phosphorus sources. Using their feeding power on superphosphate as 100, some crops showed relatively low feeding power on raw phosphate, notably red clover, wheat, oats, corn, timothy, and soybean. Others showing relatively high feeding power are rape, alfalfa, rye, buckwheat, red top, red sorrel, and sweet clover.

According to Bailey (1) in the Alabama Cullars rotation experiment, rock phosphate has produced approximately the same yields of corn and oats as superphosphate but 82 pounds less of seed cotton per acre for the period of 1911 to 1927. In these tests superphosphate was applied at the rate of 544 pounds and rock phosphate at the rate of 1088 pounds per acre.

It has been shown that raw rock phosphate is most effective when used on soils which are not high in lime. Mooers, for example (9, p. 17), draws attention to the findings of the Tennessee Agricultural Experiment Station relative to rock phosphate. He states "This Station has found repeatedly, in different parts of the state, that rock phosphate could be used with profit especially where no liming was done." It should be noted, however, that on unlimed soil in the Crossville experiments it yielded low margins of profit for oats and hay but did better when corn and potatoes were used as the plant indicators. On other lands which were unlimed, rock phosphate gave results superior to those derived from the use of acid phosphate.

Salter and Barnes, moreover (10, p. 48), in their summary of the results of numerous field experiments, mainly on Wooster and Canfield silt loams, conclude "In general it appears that the efficiency of all phosphate fertilizers on these soils decreases as the soil reaction approaches alkalinity." "The phosphorus of rock phosphate had an efficiency of around 40 per cent for both grain crops and clover on unlimed land. With repeated liming to about pH 7.5 its efficiency dropped to 10 per cent or less for the same crops. Its efficiency for sweet clover at about pH 7, was a little above 50 per cent." Tennessee brown rock phosphate was employed in these experiments.

Fraps (4) conducted pot tests in which corn was employed as the growth indicator. He found colloidal phosphate when used on two alkaline soils and Tennessee brown rock phosphate on one alkaline soil to be valueless. Where the former was applied to each of five acid soils, however, its efficiency in comparison with superphosphate on soil type one was 37, soil type two 30, soil type three 37, soil type four 69, and soil type five 120 per cent. That of Tennessee phosphate on soil type one was 34, soil type three 16, and soil type five 95 per cent.

Haskins (5) reports results of pot tests in which the relative availability of colloidal phosphate, Tennessee brown rock phosphate, and superphosphate, for the production of Dwarf Essex rape, were compared. They were applied to the soil in what he considered to be the minimum, optimum, and double optimum amounts. The average relative availability of the former in comparison with superphosphate was 0, 22.02, and 62.5 per cent for the former, and for the latter 0, 13.7, and 28.3 per cent respectively.

MATERIALS AND METHODS

The phosphates were obtained from bag lot shipments. The guaranteed P_2O_5 content of lot A of colloidal phosphate was 18, lot B 22, and lot C 27 per cent, and that of the Tennessee brown rock phosphate was 34.3 per cent. Unless stated otherwise the colloidal phosphate B was employed in the plant growth tests.

The Bouyoucos (3) hydrometer method was used in making the mechanical analyses of the phosphates and the glass electrode was employed in determining the buffering capacity of them. The pH values were determined 24 hours after the addition of the hydrochloric acid and calcium hydroxide. The amount of P_2O_5 soluble in dilute acid (H_2SO_4 buffered to pH 3.0 with ammonium sulphate) was determined colorimetrically, the phosphate-acid mixture having been shaken one-half hour (11). The treated samples were washed and centrifuged inasmuch as some of the colloidal material passed through the filters upon washing, thus introducing large errors in the determinations. Corn (*Zea mays* L. var. Golden Bantam), tomato (*Lycopersicon esculentum* Mill.), tobacco (*Nicotiana tabacum* L. var. Turkish), rye grass (*Lolium perenne* L.), Japanese millet (*Echinochloa fumentocea* Link), white sweet clover (*Melilotus alba* Mill.), and buckwheat (*Fagopyrum esculentum* Moench.) were employed in the growth studies.

Unless otherwise stated glazed jars eight and one-quarter inches in diameter and two-gallon capacity were used as containers in the greenhouse tests. The pH value of Gloucester loam employed was 5.20, Merrimac loam 5.17, and that of Illinois black clay loam was 5.34. The phosphates were mixed with three inches of soil for all crops in the greenhouse tests with the exception of rye grass, where they were mixed with two inches of soil. The field tests were carried on in galvanized iron rims two feet in diameter and eight inches deep. The surface soil was removed and the rims sunken into the subsoil. The Gloucester loam soil was thoroughly mixed before it was placed in the rims. Unless otherwise stated the phosphates were mixed with four inches of soil. Three tobacco plants were set in each rim and the same weight of buckwheat sown in each after the tobacco was harvested. Some of the tobacco plants which were injured by leaf spot were not harvested.

RESULTS

Laboratory investigations. The results of physical analyses obtained from use of the Bouyoucos hydrometer method are given in Table I. It is to be noted that the colloidal phosphates are low in sand and silt and carry high percentages of colloids. The ground Tennessee rock phosphate is higher in sand and silt and much lower in the finer fractions than are the colloidal phosphates.

The buffering capacity of colloidal phosphate B and Tennessee rock

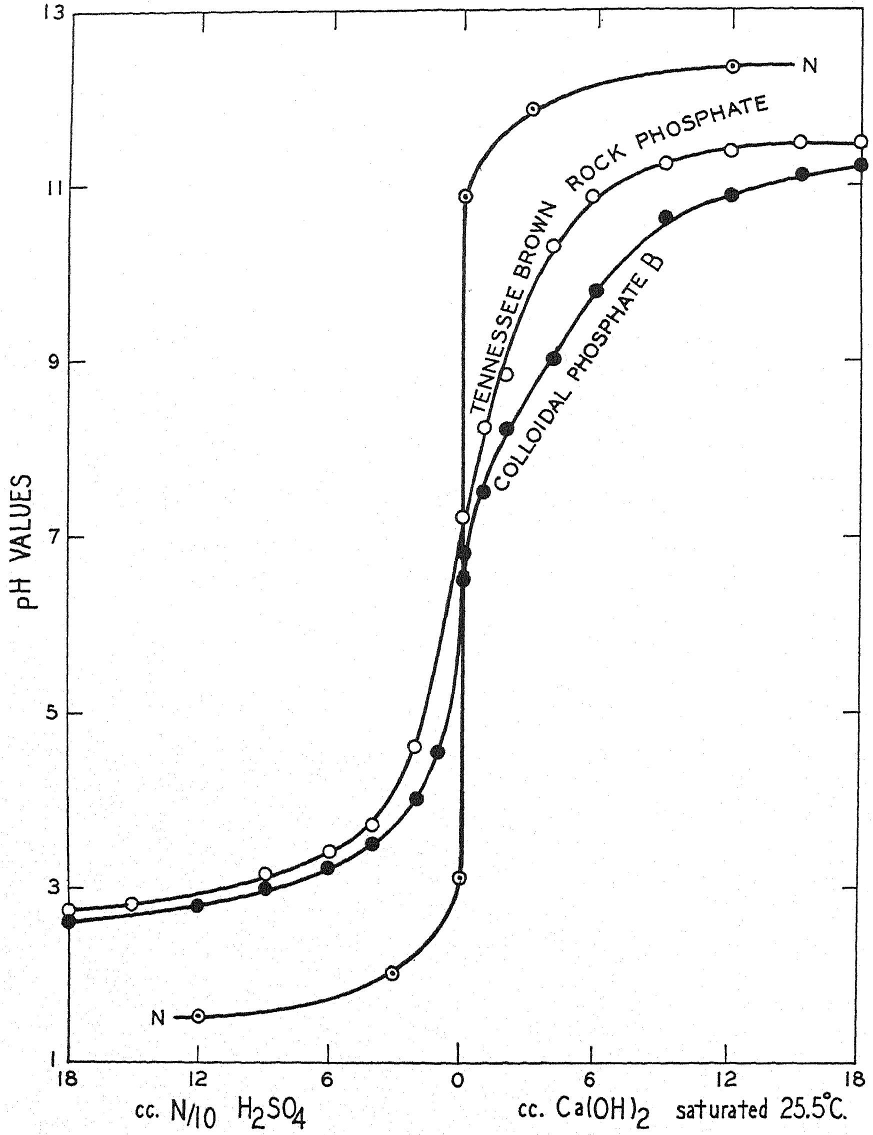

FIGURE 1. Buffering curves of colloidal phosphate B and Tennessee brown rock phosphate.

TABLE I

Mechanical Composition of Phosphates. Results Expressed as Percentage Dry Weight Basis

Phosphate	Colloidal			Tennessee
	A	B	C	
Sand 1.0 to 0.05 mm.	2.1	2.4	4.2	14.8
Silt 0.05 to 0.005 mm.	16.9	15.8	18.6	62.6
Clay 0.005 to 0.000 mm.	81.0	81.8	77.2	22.6
Finer clay 0.002 to 0.000 mm.	79.7	76.7	75.5	19.2

phosphate was determined. The results obtained are given in Figure 1. The colloidal phosphate is somewhat more highly buffered than is the Tennessee rock phosphate, the differences being greater on the alkaline than on the acid side.

The total and dilute acid soluble P_2O_5 (H_2SO_4 buffered to pH 3 with $(NH_4)_2SO_4$) in colloidal phosphate B were determined. It was found necessary to have a wider ratio of acid to phosphate than is employed with soils in order to maintain the original pH value of the extracting liquid as illustrated by the data in Table II. According to the data in Table III, the P_2O_5 content of the silt fraction of the colloidal phosphate is somewhat greater than is that of the colloidal fraction and the original sample.

TABLE II

Effect of Ratio of Dilute Sulphuric Acid to Colloidal Phosphate B on pH Value and Extractable P_2O_5

Ratio of colloid to acid	pH value	Extractable P_2O_5, %
1 to 200	4.42	1.1
1 to 500	4.34	2.7
1 to 1000	4.10	5.7
1 to 2000	3.72	11.0
1 to 4000	3.31	16.3
1 to 8000	3.08	17.0

TABLE III

Composition of Phosphates

Phosphate	CaO %	MgO %	P_2O_5 %	Acid extractable P_2O_5, %
Colloidal phosphate B	27.34	0.86	24.07	17.0
Silt fraction "	—	—	30.03	18.8
Colloidal fraction "	—	—	26.75	28.5
Tennessee rock phosphate	45.1	0.04	34.71	28.5

Plant growth tests. The results derived from the greenhouse tests in which corn, millet, white sweet clover, tomato, and rye grass were employed as the growth indicators are summarized in Tables IV, V, and VI, and illustrated by Figure 2. The field results obtained are presented in Tables VII and VIII. The statistical method followed in analyzing the data is given in Table IV.

According to the data in Table V and Figure 2 A the P_2O_5 in the colloidal fraction of the colloidal phosphate had a higher availability to millet than did that in the silt fraction. It should be recalled that the amount of the former fraction is much greater in this phosphate than is the latter, or silt fraction.

TABLE IV

CORN, GLOUCESTER LOAM

Cultural treatment	Yield, fresh wt., grams			Total
Control, no treatment	62	60	58	180
2.5 g. P_2O_5 as colloidal phosphate	98	103	84	285
5.0 g. P_2O_5 as colloidal phosphate	117	120	110	347
7.5 g. P_2O_5 as colloidal phosphate	125	114	116	355
Control, no treatment	65	65	51	181
2.5 g. P_2O_5 as Tennessee phosphate	88	94	86	268
5.0 g. P_2O_5 as Tennessee phosphate	120	108	116	344
7.5 g. P_2O_5 as Tenneseee phosphate	123	117	113	353

Analysis of Variance

Item	Degrees of freedom	Sum of squares	Mean square	Ratio
Materials	1	19	19	0.5
Amounts	3	12898	4299	*11.2*
Interaction	3	31	10.3	0.3
Error	16	614	38.4	
Total	23	13562		

TABLE V

AVAILABILITY OF SILT AND COLLOIDAL FRACTION OF COLLOIDAL PHOSPHATE B. PLANT GROWTH INDICATOR MILLET

Cultural treatment	Dry weight in grams				Total, g.
No phosphate	5.6	5.5	6.5	6.2	23.8
3 g. P_2O_5 in silt	51.5	62.5	48.5	48.6	211.1
3 g. P_2O_5 in colloids	78.5	79.5	75.8	73.4	307.2

It is to be noted from Table VI that corn (Fig. 2 B), millet, and white sweet clover grown in Gloucester loam responded to each amount of colloidal phosphate and Tennessee rock phosphate. The first two increments increased the yield of corn and millet markedly and the heaviest applica-

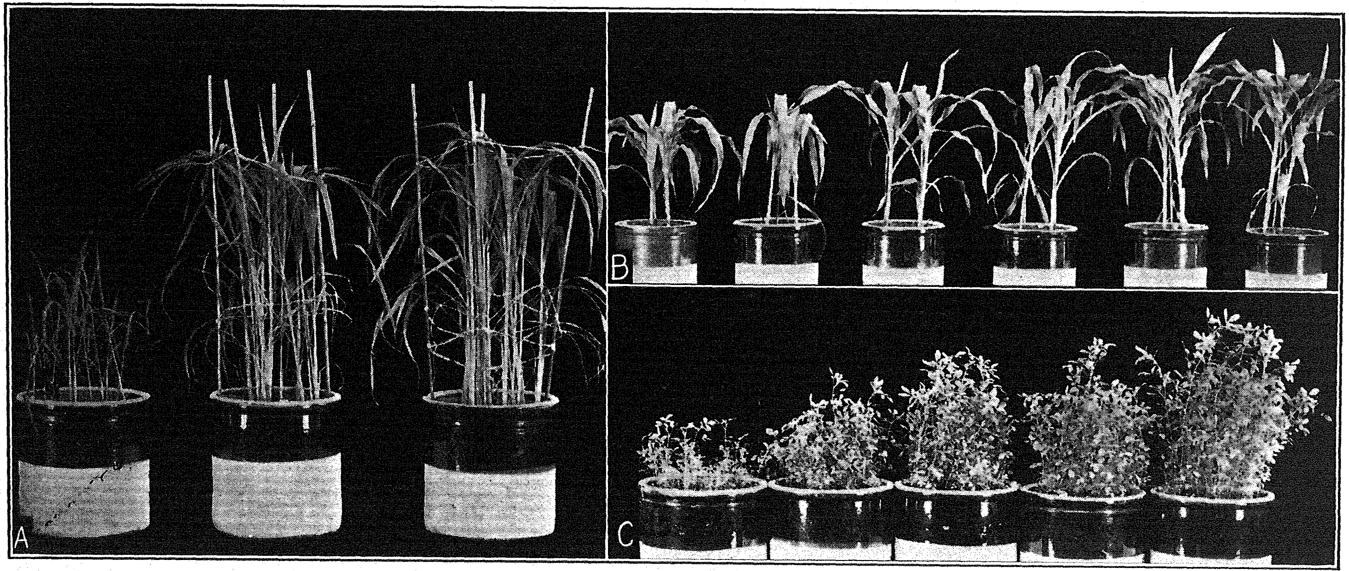

Figure 2. A. Millet growing in Gloucester loam soil. (Left) control; (Center) 1 g. P_2O_5 in silt from colloidal phosphate; (Right) 1 g. P_2O_5 in colloids from colloidal phosphate. B. Corn growing in Gloucester loam. Left to right: (1) and (2) controls; (3) 2.5 g. P_2O_5 as colloidal phosphate; (4) 2.5 g. P_2O_5 as Tennessee phosphate; (5) 5.0 g. P_2O_5 as colloidal phosphate; (6) 5.0 g. P_2O_5 as Tennessee phosphate. C. Sweet clover growing in Gloucester loam. Left to right: (1) no treatment; (2) 2.2 g. P_2O_5 in colloidal phosphate; (3) 4.4 g. P_2O_5 in Tennessee phosphate; (4) 4.4 g. P_2O_5 in colloidal phosphate; (5) 8.8 g. P_2O_5 in colloidal phosphate.

tion, or 8.8 grams of P_2O_5, also resulted in larger yields of white sweet clover than was derived from smaller amounts of it. The differences in the availability of the colloidal phosphate and the Tennessee phosphate as measured by crop response were not significant.

TABLE VI

GROWTH TESTS WITH COLLOIDAL AND TENNESSEE PHOSPHATES

Culture	P_2O_5 carrier	Amount of P_2O_5 and av. yield in grams per pot				No. of replicate pots	Standard error
		0	2.5	5.0	7.5		
Gloucester loam—corn	Colloidal	60.0	95.0	115.7	118.3	3	3.6
	Tennessee	60.3	89.3	114.7	117.7	3	
		0	2.5	5.0	7.5		
Gloucester loam—millet	Colloidal	5.9	31.3	46.0	47.0	3	2.6
	Tennessee	6.8	30.0	46.3	49.7	3	
		0	2.2	4.4	8.8		
Gloucester loam—white sweet clover	Colloidal	13.3	23.6	33.8	49.3	6	2.4
	Tennessee	15.0	26.1	30.5	43.2	6	
		0	2.2	3.1	6.2		
Illinois black silt loam—tomato	Colloidal	42.4	79.4	96.1	99.1	5	2.8
	Tennessee		77.8	100.6	106.0	5	
		0	1.0	2.0	4.0		
Illinois black silt loam—white sweet clover	Colloidal	40.4	56.5	80.0	85.6	4	2.1
	Tennessee	39.9	48.8	78.9	82.6	4	
		0	2.2	4.4			
Merrimac loam—millet	Colloidal	2.6	57.0	80.4		4	2.5
	Tennessee		57.5	85.5		4	
		0	4.4	8.8			
Merrimac loam—rye grass	Colloidal B	8.0	13.1	16.1		4	1.1
	Colloidal A		14.1	15.6		4	
	Tennessee		14.2			4	

The yields of tomato plants grown in Illinois black silt loam were augmented by the addition of 2.2 and 3.1 grams of P_2O_5 but no further increase was derived by the application of 6.2 grams. The growth of white sweet clover was enhanced by treating the soil with 1, 2, and 4 grams of P_2O_5 but the largest increase was derived from the first two amounts (Fig. 2 C). The two carriers of phosphorus did not differ significantly in their effects on yields of these crops.

Merrimac loam responded strikingly and within the limits of experimental error to the same extent, to each of the phosphorus carriers in the production of millet. Similar relationships were obtained from the growth of rye grass. Thus these results are the same as those reported by Fraps (4) and Haskins (5).

According to the data in Table VII the yield of the leaves of tobacco plants in field tests was increased greatly by application of colloidal phosphate at the rate of 1500 pounds per acre. The Tennessee rock phosphate afforded results which were not significantly different from those derived from the colloidal phosphate. It is notable that colloidal phosphate placed five inches below the surface of the soil was less effective than it was when mixed with the first four inches. Although the data are not presented, similar results were derived from cultures in the greenhouse.

TABLE VII

Tobacco Field Tests, Gloucester Loam

Cultural treatment	Dry weight of leaves per plant, grams												Av. wt. per plant, g.
No phosphate	7.7	5.5	5.6	9.9	7.5	6.0	5.0	4.9	6.6	6.1	—	—	6.48
1500 lbs. colloidal phosphate per acre	13.3	9.9	8.2	16.8	10.0	10.6	12.9	10.5	13.0	12.0	—	—	11.72
1500 lbs. colloidal phosphate per acre below 5″	6.6	12.4	8.4	8.7	7.8	9.8	6.4	6.4	12.3	12.8	11.2	—	9.34
P_2O_5 in Tennessee, 1500 lbs. colloidal phosphate per acre	8.1	11.5	6.0	9.0	10.9	13.8	7.9	10.3	11.7	16.6	—	—	10.58
2000 lbs. colloidal phosphate per acre	18.7	12.3	11.5	15.3	9.4	16.5	11.3	12.8	12.1	14.7	—	—	13.46
4000 lbs. colloidal phosphate per acre	19.7	14.9	12.1	16.3	14.8	15.5	18.6	12.2	14.3	14.6	11.9	12.1	14.75

TABLE VIII

Buckwheat Field Tests, Gloucester Loam. Fresh Weight in Grams

Control	334.5 lbs. per acre of P_2O_5 as colloidal phosphate	334.5 lbs. per acre of P_2O_5 as Tennessee rock phosphate
229	337	384
235	388	294
232	375	331
228	400	380
Totals 924	1500	1389

The results of field trials derived from buckwheat are found in Table VIII. Here again the addition of the phosphates increased the yield of the plant growth indicator to the same extent.

It should be noted that the various growth tests do not reveal whether or not the minor elements present in the colloidal phosphate were of value to the plants inasmuch as soils known to be deficient in one or more of them were not available for use.

SUMMARY

Samples of Florida pond or colloidal phosphates employed in these studies were found to be high in the colloidal fraction and more strongly buffered than finely ground Tennessee brown rock phosphate, especially on the alkaline side.

There was more total and dilute sulphuric acid extractable P_2O_5 in the silt fraction than there was in the colloidal fraction, yet a given amount of P_2O_5 applied to the soil in the latter produced a larger yield of millet than it did when added in the former.

Large increases in the yield of plants resulted from the addition of colloidal phosphates to each of three acid phosphorus-deficient soils. Whether tested in the greenhouse or in the field, colloidal phosphate and Tennessee brown rock phosphate did not differ significantly as a source of P_2O_5. The differences between the results derived from greenhouse and field tests in which the P_2O_5 in colloidal phosphate and Tennessee brown rock phosphate was compared were not significant. Colloidal phosphate was more readily available when mixed with four inches of soil than it was when placed below five inches of it.

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DISINTEGRATION OF THE CELL MEMBRANE OF THE COTTON FIBER BY A PURE CULTURE OF BACTERIA

FLORENCE E. HOOPER[1]

The cell wall of the cotton fiber consists of an outer limiting non-cellulosic membrane, or cuticle, and an inner membrane composed of crystalline and non-crystalline materials. The crystalline and most abundant constituent is present in the form of uniform ellipsoidal particles of cellulose, arranged end to end to form the spirally wound fibrils of the wall. The non-crystalline materials constitute a continuous phase which surrounds, and serves as a cementing material for the particles and fibrils (4). This structure makes it of interest to determine whether bacteria which disintegrate the cotton fiber do so by means of a selective action on one of the wall constituents or by a general digestion of all of them. The bacteria used in these studies were isolated in pure culture by means of an inorganic medium with filter paper as the sole source of carbon. Microscopic observations made during the course of disintegration of cotton fibers by these particular microorganisms have shown that a general digestion of the wall is involved. Since the bacteria studied were closely related and their action on the fiber appeared to be identical, the present report is confined to that member of the group which was studied most exhaustively.

BACTERIOLOGICAL STUDIES

ISOLATION

The organism used in the present experiments was isolated from greenhouse soil at the Boyce Thompson Institute in the spring of 1937. The soil was collected under aseptic conditions and suspended in sterile distilled water. A few drops of this suspension were used to inoculate a tube containing the following synthetic medium recommended by Dubos (3, p. 227):

$NaNO_3$	0.50 g.
K_2HPO_4	1.00
$MgSO_4 \cdot 7H_2O$	0.50
KCl	0.50
$FeSO_4 \cdot 7H_2O$	0.01
Distilled water	1000
Filter paper strips	

Five to ten cc. of this solution were added to each test tube and a piece of filter paper was partly immersed in the liquid. The pH of the medium was

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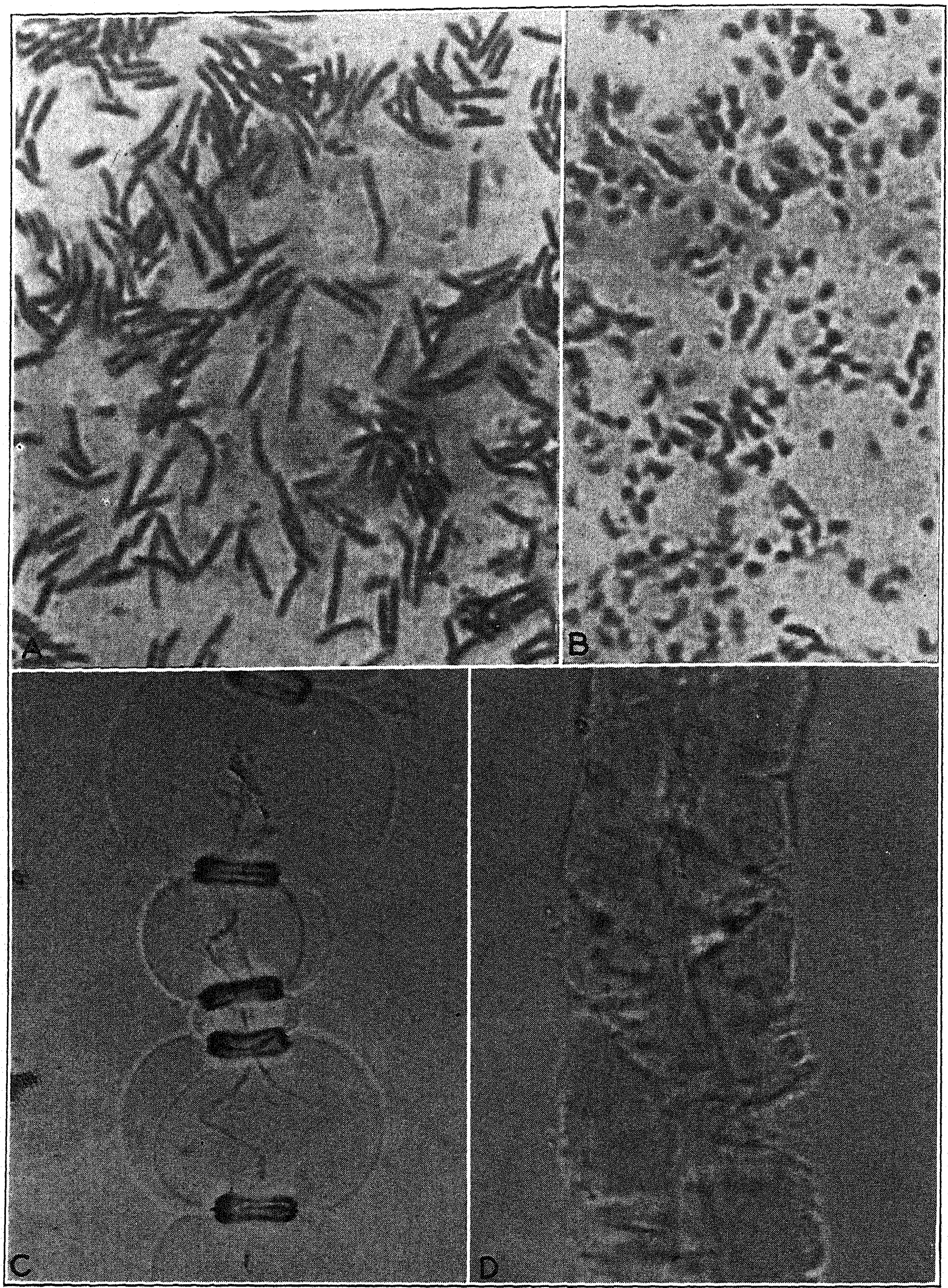

FIGURE 1. (For description see legend on opposite page.)

7.8. As soon as growth occurred as evidenced by gelatinization or shredding of the paper, the culture was used to inoculate fresh medium. This dilution-enrichment procedure was repeated until the culture contained only one or two contaminating organisms. Final purification was accomplished by streaking on starch agar plates and fishing the resulting colonies to the filter paper medium. A pure culture was obtained in less than three months after collection of the soil sample. The stock culture has been maintained by biweekly or weekly transfers on the filter paper medium. The ability of the organism to decompose cellulosic materials appears unchanged after more than a year and a half in pure culture.

DESCRIPTION

Descriptive data were obtained by means of the methods given in the Manual of Methods of the Society of American Bacteriologists (10, Leaf. 5, 6th ed., 20 pp.). Observations on nitrate reduction, oxygen requirements, temperature relations, pH change during growth, and utilization of ammonia nitrogen were limited to cultures in filter paper medium. The carbohydrates tested were used in 0.1 per cent and 0.5 per cent concentrations in place of filter paper in Dubos medium. The carbohydrate solutions and the medium were sterilized separately and mixed under aseptic conditions. All pH determinations were made colorimetrically (10, Leaf. 9, 6th ed., 7–13).

Morphology. Slender rods, straight or slightly curved, usually occurring singly (Fig. 1 A). Motile. Gram negative. No spores. Old cultures frequently show coccoid forms (Fig. 1 B).

Colonies on starch agar. Small, thin, grayish-white, translucent, slightly convex colonies with smooth margin and moist smooth surface.

Nutrient gelatin. No growth.

Nutrient agar slant. No growth.

Potato agar slant. No growth.

Purple milk. No growth.

Nutrient broth. No growth.

Nutrient broth and filter paper. Growth was indicated in 36 to 48 hours by slight turbidity and shredding or fragmentation of filter paper at the surface of the medium.

Dubos filter paper medium. Growth was obtained similar to, but usually heavier than that on nutrient broth and filter paper.

FIGURE 1. (A) Bacteria from 24-hour culture on starch agar, stained with crystal violet (×1840, enlarged to 2750). (B) Bacteria from 1-week culture on starch agar, stained with carbol fuchsin (×1840, enlarged to 2650). (C) Cotton fiber from uninoculated control, swollen in 15 per cent sodium hydroxide and carbon disulphide (×200, enlarged to 350). (D) Fiber from 4-day culture, swollen in 15 per cent sodium hydroxide and carbon disulphide (×200, enlarged to 300).

Utilization of ammonia nitrogen. A modified Dubos medium[2] containing ammonium sulphate in place of sodium nitrate supported growth as did the regular Dubos filter paper medium.

Nitrate reduction. Nitrates in Dubos medium were reduced to nitrites but not to ammonia (10, Leaf. 6, 5th ed., 3–15).

Oxygen requirements. Strictly aerobic.

Temperature relations. Optimum growth was obtained at 30° C., no growth at 37° C.

Change of pH during growth. A drop of about 0.15 pH occurred during the first week of growth on Dubos medium. This was followed by a gradual rise until the third or fourth week, when a constant value about 0.3 pH higher than that of the original medium was reached.

Utilization of carbohydrates. Growth was obtained with glucose, fructose, xylose, galactose, maltose, lactose, cellobiose, sucrose, salicin, dextrin, starch, pectin, and amyloid from nasturtium endosperm in Dubos medium. No gas was produced. No evidence of acid production was obtained in media containing carbohydrates in 0.1 per cent concentration. Of those tested in 0.5 per cent concentration in Dubos medium, namely, glucose, maltose, lactose, cellobiose, starch, pectin, and sucrose, acid was produced in all except sucrose.

Attack on filter paper in presence of other carbohydrates. Filter paper was attacked in usual manner in presence of 0.1 per cent of all carbohydrates listed above, and 0.5 per cent sucrose. Five-tenths per cent glucose, lactose, maltose, cellobiose, starch, and pectin either completely inhibited or greatly retarded the attack on paper.

Cellulose acetate. No growth occurred in Dubos medium containing cellulose acetate rayon in place of filter paper.

Cellulose from hydrochloric acid-disintegrated cotton fibers (5). Slow growth was obtained on Dubos medium containing the cellulosic residue from hydrochloric acid-disintegrated cotton fibers in place of filter paper.

CLASSIFICATION

This organism does not appear to be identical with any listed in Bergey's Manual of Determinative Bacteriology, fourth edition (1). It is probably closely related to the aerobic forms described by Dubos (3); however, from available data it is impossible to say whether it is identical

[2]

$(NH_4)_2SO_4$	1.00 g.
K_2HPO_4	2.00
$MgSO_4 \cdot 7H_2O$	0.50
KCl	0.50
$FeSO_4 \cdot 7H_2O$	0.01
Distilled water	1000
Filter paper strips	

with any of them. No attempt at classification has been made. This culture has been designated as No. 1 in the laboratory collection.

DISINTEGRATION OF COTTON FIBERS BY THE ORGANISM

Two-tenths gram portions of raw cotton fibers, Super Seven variety (*Gossypium hirsutum* L. Strain 4), were immersed in 100 cc. of Dubos medium and sterilized for 20 to 30 minutes at 15 lbs. pressure in the autoclave. The flasks were inoculated with 3 cc. of 3- to 6-day-old Dubos filter paper cultures. Care was taken to introduce a minimum of paper fibers into the flasks. The cultures were incubated at room temperature or at 30° C. Growth was indicated a few days after inoculation by the appearance of a fine turbidity which differed from the small amount of solid matter always present in the medium in that it did not settle on standing. At about the same time the cotton appeared softer and somewhat cream-colored. As growth continued, the fiber mass showed an increasing tendency to break up into short fragments. Complete digestion of fibrous material has not been observed even after eight months' incubation. The weights of residual fibers obtained from several cultures of various ages are shown in Table I.

TABLE I

COTTON FIBERS DIGESTED BY BACTERIA DURING VARIOUS GROWTH PERIODS

Growth period	Cotton in medium in grams	Cotton in culture at end of experiment in grams*	Cotton digested by bacteria	
			Grams	Per cent
Uninoculated control	0.200	0.199	—	—
8 days	0.201	0.159	0.042	21
2 weeks	0.200	0.149	0.051	26
4 weeks	0.200	0.123	0.077	38
6 weeks	0.200	0.103	0.097	48
24 weeks	0.200	0.043	0.157	78

* Weight of residual material in cultures retained by a 1G3 Jena filter.

Microscopic observations were made on cultures of various ages up to six months. Several mounts were examined from each flask opened. In order to guard against the use of contaminated material, flasks once opened were discarded. Fibers were mounted directly in the culture medium to avoid loss of small fragments or particles. Control observations were made on cotton fibers from flasks of media which had been autoclaved but not inoculated. Observations were made on unstained fibers and on fibers swollen with sodium hydroxide, sodium hydroxide and carbon disulphide, and sulphuric acid and iodine. The latter reagents were especially useful in differentiating between masses of bacteria and fiber fragments which in the untreated state sometimes closely resembled each other. When

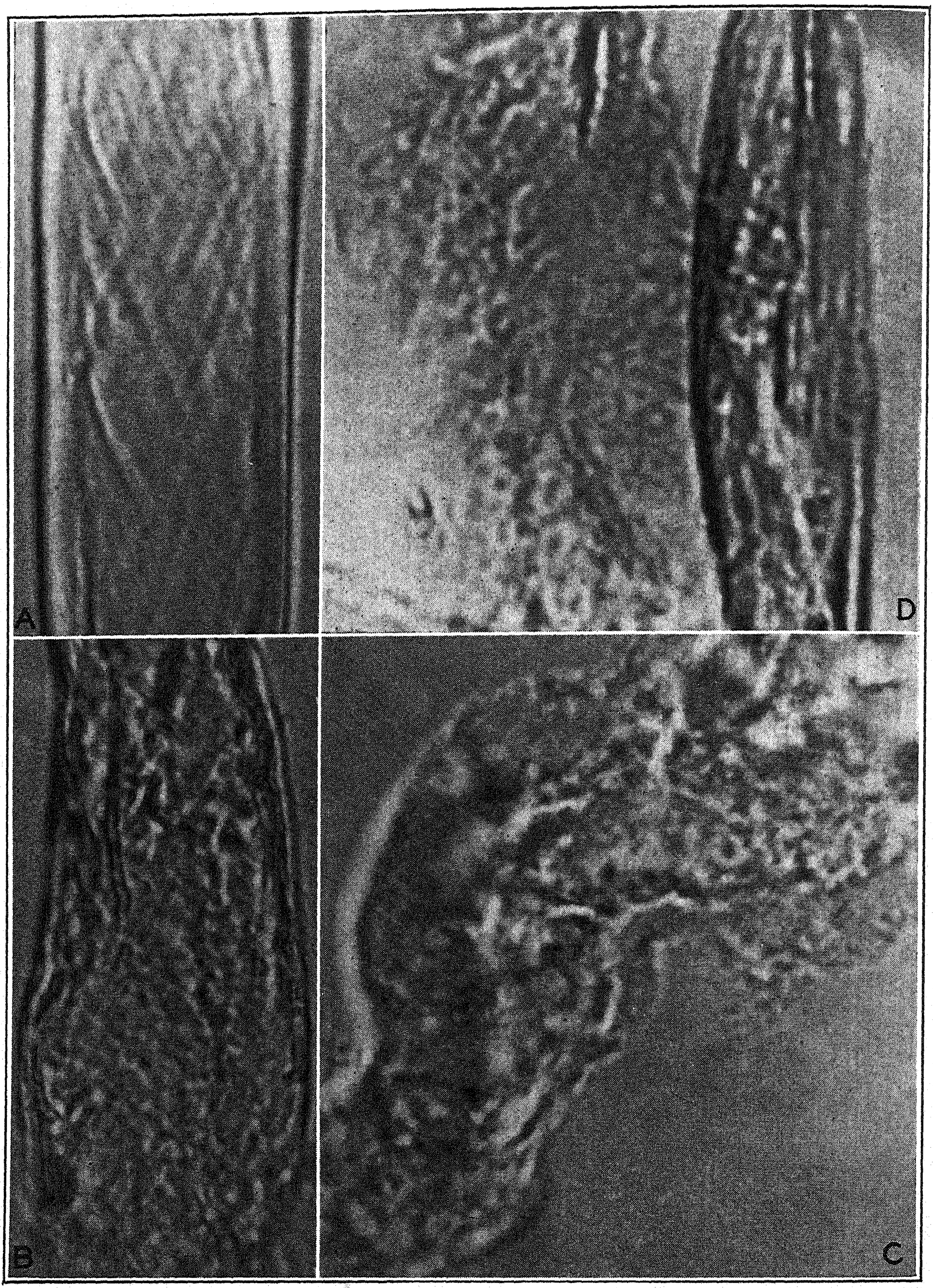

FIGURE 2. (For description see legend on opposite page.)

treated with iodine and sulphuric acid, the bacteria gave a brown and the cellulose a blue color.

Examination of mounts from young cultures, four or more days old, which showed growth but in which fragmentation of the fiber had not begun, showed a large proportion of the fibers to be slightly rough on the surface (Fig. 2 B). Fibers from uninoculated control flasks were smooth. Swelling with 15 per cent sodium hydroxide served to demonstrate more clearly this difference between fibers from cultures and the control (Fig. 2 C and A). On swelling with 15 per cent sodium hydroxide and carbon disulphide (6), fibers from the culture generally failed to show the lantern-like swelling and constricting cuticular bands characteristic of the swollen fibers from the control (Fig. 1 D and C). These observations indicate that the organism under investigation removes the cuticle and then attacks the remainder of the fiber from the surface. There seems no reason to suppose that attack does not also occur from the lumen.

As disintegration proceeded the surface of the fibers became more deeply corroded, and frequently whole sections of wall were found to be missing. The number of cellulose lamellae in the walls decreased and a general loosening of fiber structure occurred. In this condition the spirally wound fibrils of the wall were readily seen (Fig. 3 A, B, C, D, and 2 D) to consist of ellipsoidal cellulose particles as described by Farr and Eckerson (4). Sometimes all that remained of a fiber were two or three twisted fibrils (Fig. 3 C). Short fibrils and some isolated particles were also found. With the exception of the very young cultures in which absence of cuticle and slightly corroded fiber surface were the only visible evidence of attack, all flasks examined showed fibers in all the stages of disintegration described above, the older cultures showing a higher proportion of the more completely disintegrated fibers.

The loosening of fiber structure recorded above is evidence of removal of the cementing material by the action of the bacteria. While some isolated cellulose particles were always found in the culture flasks, the number of them was far too low to account for even an appreciable fraction of the cellulose particles originally present in the fiber. From this it may be concluded that this bacterium utilizes the cellulose particles as well as the cementing material of the cotton fiber.

Although all the constituents of the cell wall appear to be digested by this organism there is no reason to assume that such will be true in all cases of bacterial disintegration of the fiber. It is of interest in this connec-

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FIGURE 2. (A) Cotton fiber from uninoculated control, swollen 18 hours in 15 per cent sodium hydroxide (×900, enlarged to 1350). (B) Fiber from 11-day culture (×900, enlarged to 1350). (C) Same fiber immediately after mounting in 15 per cent sodium hydroxide (×900, enlarged to 1350). (D) Partially disintegrated fibers from 9-week culture, stained with sulphuric acid-iodine (×900, enlarged to 1350).

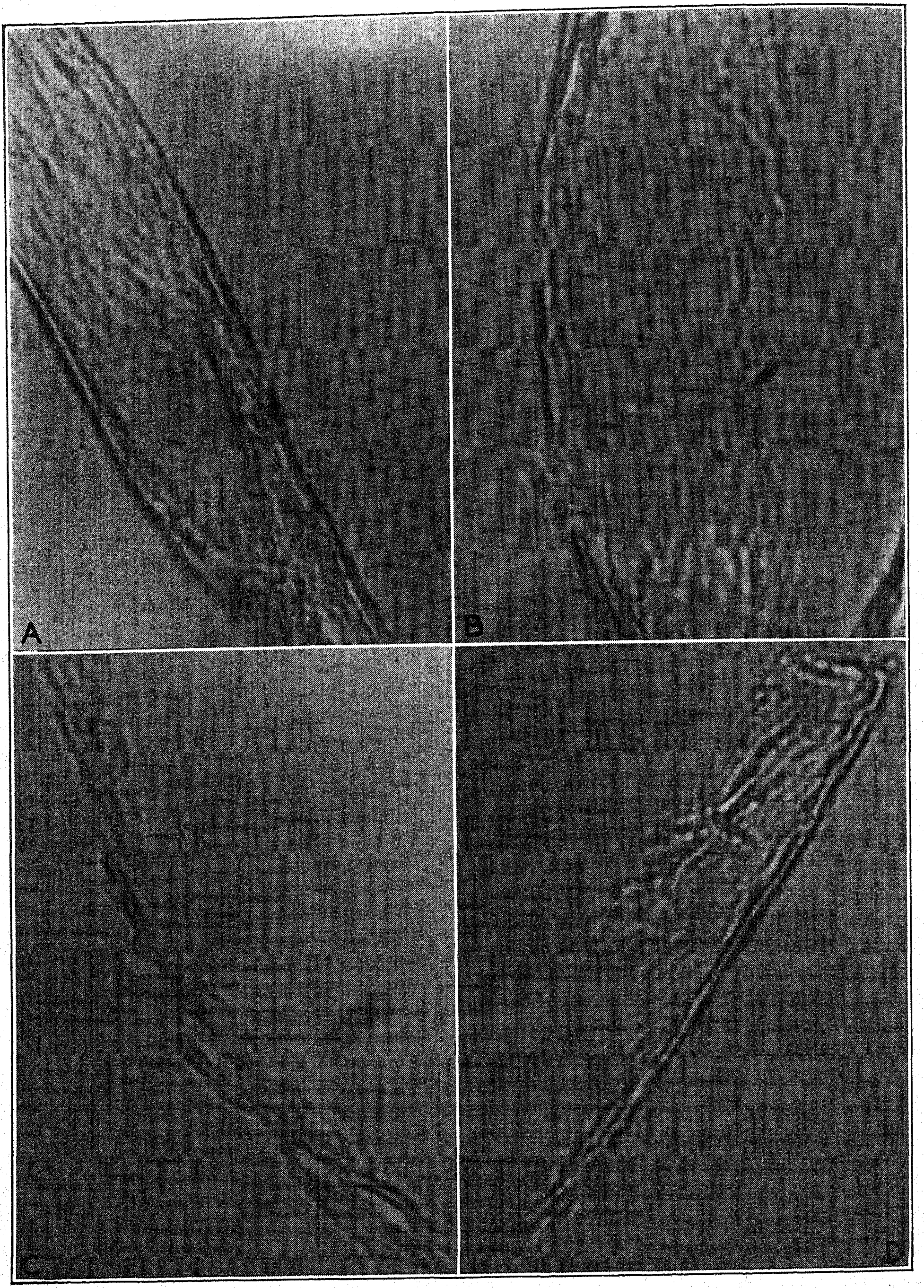

FIGURE 3. (For description see legend on opposite page.)

tion that Gulati (8) has reported a type of fiber damage due to microorganisms in which the cellulosic layers have been completely removed, leaving only the cuticle along certain lengths of the wall. The observations of Fleming and Thaysen (6, 7), Thaysen and Bunker (11), Burns (2), and Prindle (9) on cotton damaged by mixed and unidentified flora, on the other hand, indicate that microbiological attack on the surface of the fiber is of common occurrence.

SUMMARY

1. An aerobic microorganism capable of growing on inorganic medium with such cellulosic materials as filter paper and cotton as the sole source of carbon has been isolated from the soil and studied in detail.

2. The disintegration of cotton fibers by this organism has been followed microscopically. The cuticle is removed from the fiber during the early stages of attack and then both cementing material and cellulose are slowly digested. The particulate nature of the cellulose fibrils of the cotton fiber cell wall is clearly visible in the partially disintegrated fibers.

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FIGURE 3. Cotton fibers, partially disintegrated by bacteria. (A) From 24-day culture (×675, enlarged to 1000). (B) From 8-week culture (×900, enlarged to 1350). (C) From 9-week culture (×900, enlarged to 1350). (D) From 24-day culture (×675, enlarged to 1000).

A CECIDOMYID LARVA INFESTING FLOWERING STEMS OF LILIES[1]

E. P. Imle and Albert Hartzell

An insect trouble, new to lily culture, was found affecting stems of *Lilium auratum* Lindl. at Yonkers, New York on July 29, 1937. Investigation showed the stems to be heavily infested with a small cecidomyid larva (Fig. 1 C). These affected plants were growing in nursery rows among evergreens at the Boyce Thompson Institute Arboretum and ranged from four to six feet in height. The leaves and buds near the apex of the flowering stalk of nearly all these plants were blighted (Fig. 1 A and B). The youngest buds at the top of the stalk were in some cases completely killed. Some of the buds farther down the stalk were blackened at their bases or had blackened necrotic areas extending down one side of the bud causing distortion and splitting (Fig. 1 A and B). On some plants the lower buds had developed into normal flowers but on others no healthy flowers were produced as a result of the infestation. The leaves near the top of the stalk were dead and brown. Those farther down the stalk were partly necrotic and of a pale yellow or brown color.

Superficially the symptoms exhibited by infested plants might be confused with symptoms of the "crookneck" phase of the mosaic disease in *Lilium auratum*. They are alike in the top necrosis of the stem and in the blackening of the bases of the buds. The "crookneck" disease, however, causes all the leaves and buds to drop, leaving a dead stalk with the top crooked over. None of the plants with the stem larval infestation showed such symptoms.

There were no necrotic spots produced such as characterize the *Botrytis* disease. The leaf necrosis was in the nature of a general drying, such as would result from the formation of an abscission layer rather than a necrotic spot symptom as produced by *Botrytis elliptica* (Berk.) Cooke.

The cecidomyid larvae were feeding in the pith and in some instances had infested as much as the top one-third of the stem. The larvae were also found in the flower pedicels. Such infestation caused the buds to be blasted or withered. In some instances the larvae had penetrated from the pedicels into the buds, boring through the ovary tissue and half way the length of the style. Small exit holes were found on the upper part of the stem to which cocoons were often attached (Fig. 1 D).

Infested flower stalks were caged in order to rear the adult flies, which emerged August 3, 1937. Adults sent to E. P. Felt, of Stamford, Connecti-

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[1] This work was conducted under the terms of a fellowship established and supported jointly by the Boyce Thompson Institute for Plant Research, Inc. and Cornell University.

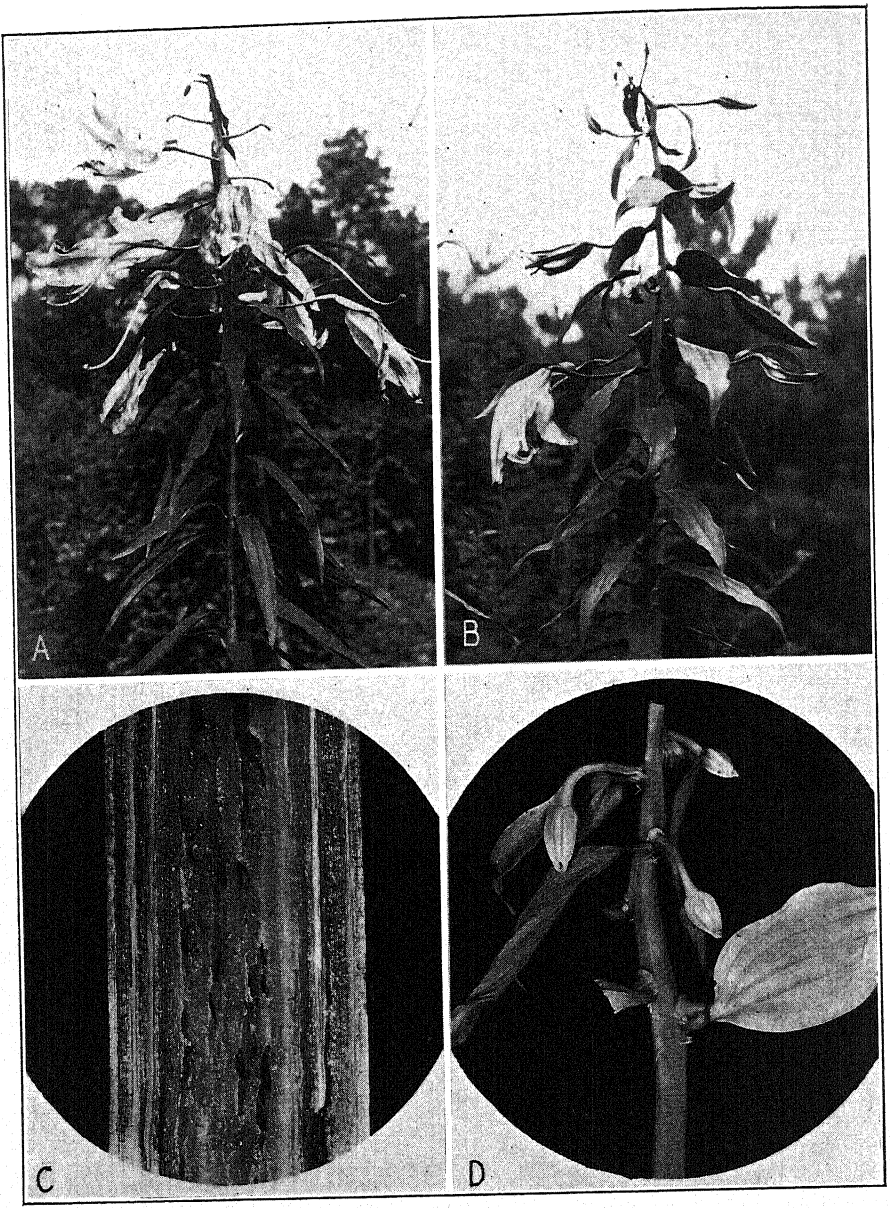

FIGURE 1. *Neolasioptera hibisci* injury to *Lilium auratum*. A. Bud pedicels and buds at top of the flowering stalk destroyed by larvae. B. Late blooming plant buds destroyed by same species of larvae. C. Orange colored larvae in pith of split stem (×3.3). D. Emergence cocoons. Note also blighted buds.

cut, were identified as *Neolasioptera hibisci* Felt. This species has been reported as causing slight enlargement of the stems of swamp rose mallow, *Hibiscus moscheutos* (1). No enlargement was observed in the present investigation with *Lilium auratum*, *L. speciosum* Thunb., *L. tigrinum* Ker., or *L. formosanum* Wallace. In his Key to the American insect galls, Felt (3, p. 171) lists this insect under the common name of mallow stem midge. Later he (2) states that the larvae occur singly or in numbers in the pith and occasionally in the outer portions of the tissues. One stalk may be inhabited with from 1 to 100 or more larvae. The eggs appear to be laid in small slits in the stem, the larvae tunneling the pith and producing enlargements approximately proportional to the degree of infestation (4). His (2) description of the larva follows: "Length 4 mm., rather stout, light yellowish. Head small; antennae uniarticulate, slender, tapering; breastbone stout, somewhat expanded apically, bidentate and with a rudimentary median tooth; skin finely shagreened; posterior extremity broadly rounded."

Later reports from A. B. Stout, of the New York Botanical Garden, indicate the same insect has been found attacking *Lilium formosanum*, *L. speciosum*, and *L. auratum* in other localities. Norma E. Pfeiffer, of Boyce Thompson Institute for Plant Research, Inc., reports receiving a specimen of *L. formosanum* infested by the same species of insect. No such infestations were found nor reported during the 1938 season.

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EFFECTS ON ANIMALS OF PROLONGED EXPOSURE TO SULPHUR DIOXIDE

F. R. Weedon,[1] Albert Hartzell, and Carl Setterstrom

The effects of sulphur dioxide on men and on animals have been the subject of considerable controversial discussion, but they have been the source of meager experimental data despite the prevalence of sulphur dioxide as an atmospheric contaminant and despite its oft-mentioned "harmful effects on human health."

As one of the gaseous products of the combustion of coal and of certain industrial operations, sulphur dioxide is discharged into the atmosphere of industrial centers in large quantities. Pincus and Stern (18) estimate that exclusive of the discharge of internal combustion engines, the amount of sulphur dioxide released into the atmosphere of New York City during 1934 averaged close to 2000 tons per day. Recent studies by the Air Hygiene Foundation (14), the National Research Council of Canada (10), and the Boyce Thompson Institute (22) reveal that measurable concentrations of sulphur dioxide prevail over large areas surrounding industrial centers and suggest that concentrations as high as three parts per million parts of air by volume (p.p.m.) are encountered in such cities as St. Louis, Pittsburgh, and New York. Data of Kehoe *et al.* (11) show that workers engaged in the manufacture of refrigerants are frequently exposed to concentrations varying from 30 to 100 p.p.m. It is likely that workers in ore smelters occasionally encounter concentrations greater than 100 p.p.m. High concentrations are also encountered in the manufacture of sulphuric acid and in the many industrial processes which use sulphur dioxide as a fumigant or as a bleaching agent.

The Meuse Valley disaster of 1930 focused attention on the possible importance of sulphur dioxide as an atmospheric contaminant. During a thick fog which covered Belgium's Meuse Valley from the first to the fifth of December, several hundred people were attacked by severe respiratory disturbances and 63 people died shortly after developing initial symptoms. Firket (4) and his co-investigators of the University of Liége conclude that the noxious gases were "SO_2 and its oxidation product H_2SO_4." Firket's data indicate that the maximum concentration which could have been reached after four days of fog was 38 p.p.m. if it is assumed that no SO_2 was removed by adsorption or oxidation.

Langrishe (13, p. 981) of the University of Edinburgh cites statistical evidence showing a relationship between death rates and atmospheric con-

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ditions in Glasgow, "the number of deaths from pulmonary and heart diseases increasing in direct proportion to the intensity and duration of smoke-fogs [usually of high SO_2 content]."

The committee on public health relations of the New York Academy of Medicine, writing on effects of air pollution on health (15, p. 753) reports, "It is probable that sulphurous fumes are the most deadly of all the gaseous constituents of smoke. In this connection Evans states: 'Sulphur compounds are very objectionable and probably more harmful than carbon compounds.' Schaefer, who has made a special study of the effects of sulphur gases on health, attributes lasting and serious results to the inhalation of these gases."

Parr (17) in his presidential address before the American Chemical Society states that the presence of SO_2 in city air is evidence of a positive and overwhelming insanitary state of the air in every large community, and he suggests "as a topic for study by the new foundation for research into the cause of common colds—the effects upon the mucous tissues of sulphur dioxide gas."

Despite these and other implicating opinions of responsible scientists, which have been echoed by the popular press (16, 24), practically no experimental data are reported in the literature on the toxicity of those concentrations of sulphur dioxide which are encountered in industrial atmospheres.

The lack of experimental data is the more surprising because the problem is of peculiar importance to industrial interests. For example, elaborate sulphur dioxide removal equipment was installed in the London Power Company's Battersea power plant because of legal restrictions imposed in part as the result of apprehensions of residents of the vicinity regarding the harmful effects of sulphur dioxide on health (30). In this country extensive research (8, 9) on methods for the removal of sulphur compounds from flue gases receives stimulus from considerations of supposed effects of these compounds on human health.

Most of the literature on the toxicity of sulphur dioxide is concerned with the treatment of one or two animals with extremely high concentrations (500–2000 p.p.m.) for short periods of time. Some of these data will be reviewed and discussed under the heading of "Discussion and Conclusions." While Kisskalt (12) performed some experiments with low concentrations and found that experimentally induced tuberculosis was aggravated by breathing small amounts of SO_2 (6.5–37 p.p.m.), his apparatus did not permit precise control of the gas concentrations, his animals were few, and his results questioned by Flury and Zernik (5, p. 143) and others because of the fact that workers on producer ovens and in sulphite cellulose factories are reported to remain free of tuberculosis and of infectious diseases of the respiratory tract.

The most important evidence in the literature on the effect of prolonged exposure to SO_2 seems to be that of Kehoe, Machle, Kitzmiller, and LeBlanc (11) who made a careful survey of workers, who had been intermittently exposed to concentrations of 30 to 100 p.p.m. of SO_2 for periods up to several years. These authors (p. 173) summarize their conclusions as follows:

"1. A significant higher incidence of nasopharyngitis, both chronic and slight, alterations in sense of smell and taste, and increased sensitivity to other irritants was found in a group of workers exposed to SO_2. These may be thought of as conditions arising from local irritation.

"2. Significantly higher incidence of abnormal urinary acidity, of tendency to increased fatigue, of shortness of breath on exertion, and abnormal reflexes was found in the exposed group. These are in the nature of systemic symptoms.

"3. There was no demonstrable association between frequency or severity of individual symptoms and frequency of heavy exposures, nor was there any relation between exposure and the frequency and severity of symptoms arising from customary exposure.

"4. A positive relation exists between frequency of exposure and the presence of certain symptoms indicative of acid accumulation, namely, increased fatigability during the period of employment, and shortness of breath on exertion.

"5. The incidence of colds is not significantly different in the two groups. The duration, however, is extended in the exposed group.

"6. In all other respects as indicated, there was no significant difference between the two groups."

A general review of the pathological symptoms of irritant gas poisoning is given by numerous authoritative works (e.g., 1, 7, 19, 26, 27). These reviews, however, deal primarily with exposures to extremely high concentrations.

The present work was undertaken to determine the toxicity of sulphur dioxide to certain laboratory animals, in the hope that the information would serve to clarify the various opinions regarding the effects of this gas on health; and in the belief that such a study of the action of a "simple" irritant gas might furnish some insight into the physiological mechanism of gas poisoning.

After the work reported in this paper was almost completed, the authors obtained a typewritten copy of an unpublished report by Vedder and Armstrong (28) of the Chemical Warfare Service dealing with experiments they had performed on the toxicity of sulphur dioxide. This work represents a great improvement over all previous experimental work and it will be reviewed in some detail under the heading "Discussion and Conclusions."

APPARATUS

The apparatus used in this study has been described in detail by Setterstrom and Zimmerman (21). It consists of duplicate sets, one for treating the animals with the sulphur dioxide, the other for controls. Each set includes a glass cabinet of special design and construction; a variable speed exhauster-blower and an orifice meter for precise control of the volume of air passing through the cabinet; a specially designed scrubber attached to the intake side of the blower to rid the air of atmospheric SO_2 before it enters the cabinet; a Bristol humidigraph for recording the temperature and relative humidity in the cabinet; a Thomas autometer and a Leeds and Northrup Micromax conductivity recorder for recording sulphur dioxide concentrations in the cabinet.

All the apparatus, except the conductivity recorders, is housed in a small greenhouse, equipped with automatic heating units. The conductivity recorders are installed in a laboratory about 20 feet from the greenhouse.

Accurate metering of the sulphur dioxide entering a cabinet is made possible by use of calibrated capillary flowmeters, a reducing regulator, and a sensitive needle valve. The gas used was purchased in style H cylinders from the Ohio Chemical and Manufacturing Company and is reported by them, in a communication dated February 28, 1939, to have a purity of at least 99.90 per cent, containing no arsenic compounds, less than 50 p.p.m. of moisture, possibly a trace of SO_3, and possibly a trace of air.

In each experimental fumigation the concentrations of SO_2 were established by means of a calibrated capillary flowmeter and a calibrated orifice meter, and were finally determined with the autometer-recorder system. In each case the calculated and observed values agreed within 5 per cent with each other.

DESIGN OF EXPERIMENTS

VERTEBRATES

Because of the prolonged exposures necessary to obtain kills with low concentrations, and because of limitations of the number of animals, it was not considered expedient to attempt to express toxicity in the conventional terms of per cent kill versus concentration, keeping time of exposure constant.

It was believed that the most information could be obtained by determining time till death of 50 per cent of the exposed animals as a function of concentration. The 50 per cent point was chosen because such a figure can be obtained with greater precision than any other time versus kill value (25). As the data tabulated by Bliss (3, p. 197) show, for example, 29 animals are required to obtain the time of 10 per cent kill with the precision resulting from use of 10 animals to evaluate the 50 per cent point.

This procedure has an added advantage in that toxicity can be expressed in terms of kill versus time of exposure in each of the separate experiments, wherein the concentration is constant.

The various experiments are semi-factorial in type. Several factors (such as exercise and intermittent exposure) were studied simultaneously. Requirements of the pathologist for sufficient tissue, limitations of space and of time, however, prevented the use of the more complex experimental layouts with exact statistical analyses such as have characterized some studies of the effects of sulphur dioxide on plants (23).

INVERTEBRATES

Insects were exposed to constant concentrations of SO_2 for varying periods of time. As in the experiments with vertebrates the 50 per cent mortality points were chosen for comparison because such a figure can be obtained with greater precision than any other time versus kill value.

EXPERIMENTAL PROCEDURE

Guinea pigs and white mice were selected as vertebrate test material because of reported differences in their response to respiratory irritants (26). Only male animals were used to avoid weight irregularities resulting from pregnancy. Grasshoppers and cockroaches were used as test material in the experiments on invertebrate animals. Control animals were used in all the experiments except No. 9 and were subjected to exactly the same conditions and treatments as the SO_2-treated animals except that they were not exposed to the gas. Exp. No. 9 was of such short duration that controls were not adjudged necessary.

The guinea pigs were weighed at least once each week, and a record was kept of their food consumption. They were continually supplied with oats and were given a daily ration of carrots or cabbage. As is usual in experimental work with guinea pigs they were not watered. Tests to determine possible toxic effects of the food exposed to SO_2 are described under Exp. Nos. 8 and 9.

The mice were watered by means of the drop-fountain method, using narrow-mouthed test tubes, and were fed "Complete ration" dog pellets daily with occasional rations of cow's milk. The manufacturer's analysis of the dog pellets lists not less than 24 per cent protein, not less than 3 per cent fat, not more than 5 per cent fibre, not less than 50 per cent carbohydrates.

Throughout these experiments a careful check was made, by means of the autometer-recorder system on the control cage, of the ammonia content of the experimental atmospheres in order to avoid masking or heightening any slight SO_2 effects. Although no actual determinations were made of the ammonia content of the animal rooms where the animals were kept before and after experimentation, bedding was changed frequently to lessen the intensity of any ammonia formation.

With vertebrates efforts were made to determine the effects on susceptibility to SO_2 of the age of the animals, pretreatment with sulphur dioxide, intermittent exposures of varying lengths of time, and exercise. The details of these various treatments will be described under the headings of the individual experiments.

In allocating the guinea pigs for the various treatments they were divided into equalized weight groups and then chosen by chance. The mice were allocated solely by chance.

The guinea pigs were exercised in a motor-driven wooden cylinder, the bases of which were perforated with screened holes. The inside diameter of the cylinder was 38.6 cm., and it revolved at the rate of 1.9 revolutions per minute. The animals were exercised two hours per day, and, therefore, walked 0.172 mile during each exercise period. The "exerciser" was shifted back and forth between the two cabinets daily except Sunday, in order to exercise controls as well as treated guinea pigs. The animals were not exercised on Sundays. No attempt was made to exercise the mice because of the difficulty of building an exerciser in which they could neither cling to the sides nor slide along the bottom.

During the course of each experiment careful observations of the condition of the vertebrate animals were made at frequent intervals and noted on a "log-sheet." Upon death of an animal the body was removed to a 10° C. cold room to await autopsy, if immediate autopsy was not possible. Pathological findings are to be reported in detail in a subsequent paper and will be mentioned only briefly in the present report.

Although the history of each vertebrate animal has been traced through the many hundreds of pages of "log-sheet" observations, it was not felt that these voluminous data were important enough to publish in detail. The general findings are summarized and exceptions are noted under the headings of the various experiments.

The experimental procedure for invertebrate animals will be described in the section entitled "Invertebrates" (page 314).

EXPERIMENTAL RESULTS

VERTEBRATES

The effects of sulphur dioxide on those guinea pigs which were exposed continuously are briefly summarized in Table I. Similar data for the white mice are given in Table II. It should be noted that the animals were kept in the gas until 50 per cent or more of the animals had died and then were transferred to a sulphur dioxide-free atmosphere, except in those cases where the experiments were discontinued before the 50 per cent point was reached because no progressive symptoms developed after a considerable period of gas treatment. Toxicity curves for those experiments in which there was an appreciable kill are given in Figures 1 and 2. These experiments are summarized in the concentration-time curves of Figure 3.

TABLE I

SUMMARY OF SO_2 TOXICITY EXPERIMENTS ON GUINEA PIGS; ALL ANIMALS EXPOSED CONTINUOUSLY

Exp. No.	Av. SO_2 conc. (p.p.m.)	Length of gas treatment (hrs.)	No. animals exposed to SO_2	No. animals dead at end of treatment	Length of exposure to SO_2 of animals that died in gas (hrs.)	Total No. animals dead 2 wks. after treatment
1	63·7	360	10	5	219, 244, 288, 353, 360	5
2	10·7	720	10	0	—	2*
3	33·0	984	14	1†	262	3**
4	25·9	576	22	7†	22, 232, 278, 322, 329, 353, 460	7
5	25·3	1137	10	0	—	7*
6	112	113	8	4	54, 104, 107, 113	7
7	127	138	8	6	69, 96*, 98*, 98*, 119, 138	8
7	131	154	8	4	72, 120, 132, 146	5
8	299	89	8	4	60, 69, 73, 86	8
9	1039	24	20	14	11·9, 11·9, 12·5, 12·5, 12·5, 12·6, 14·6, 17·6, 17·6, 18·4, 19·2, 19·6, 21·0, 23·5	20

* Sacrificed for autopsy.
** Two sacrificed for autopsy.
† Pathological findings show death due to epizootic pneumonia.

TABLE II

SUMMARY OF SO_2 TOXICITY EXPERIMENTS ON MICE; ALL ANIMALS EXPOSED CONTINUOUSLY

Exp. No.	Av. SO_2 conc. (p.p.m.)	Length of gas treatment (hrs.)	No. animals exposed to SO_2	No. animals dead at end of treatment	Length of exposure to SO_2 of animals that died in gas (hrs.)	Total No. animals dead 2 wks. after treatment
2	11·2	173	10	5*	156, 156, 156, 167, 173	5
2	10·4	361	10	0	—	2**
3	33·0	984	20	8*	506, 602, 627, 656, 659, 777, 779, 888	10†
4	25·9	576	29	12*	192, 234, 246, 254, 254, 254, 254, 254, 261, 556, 556, 556	12
5	25·3	1137	15	0	—	3††
6	109	238	12	0	—	2‡
7	151	847	12	6	249, 405, 421, 630, 814, 847	6
8	300	45	12	4	28, 37, 39, 45	9†
8	288	69	12	7	46, 47, 48, 48, 59, 63, 64	11
9	907	4·3	16	9	1·9, 2·5, 3·1, 3·4, 3·9, 4·1, 4·1, 4·2, 4·3	13

* Pathological findings show death due to epizootic enteritis.
** One sacrificed for autopsy.
† Two sacrificed for autopsy.
†† Three sacrificed for autopsy.
‡ Two animals died as result of accidental exposure to a very high concentration of SO_2.

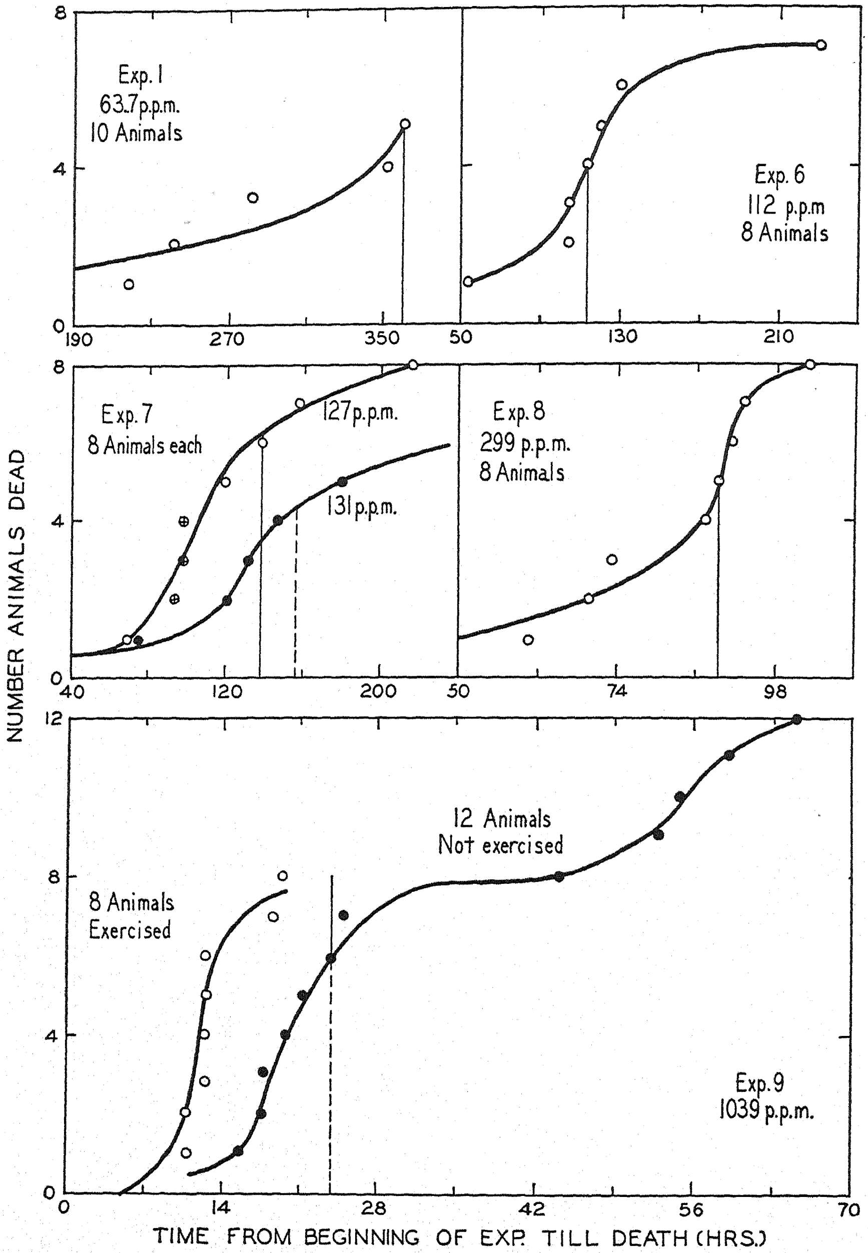

FIGURE 1. Mortality versus time toxicity curves for guinea pig experiments. The vertical lines to the curves indicate the times at which the SO_2-treatments were discontinued. The circles with crosses in the curve for Exp. No. 7 indicate animals which were sacrificed for autopsy.

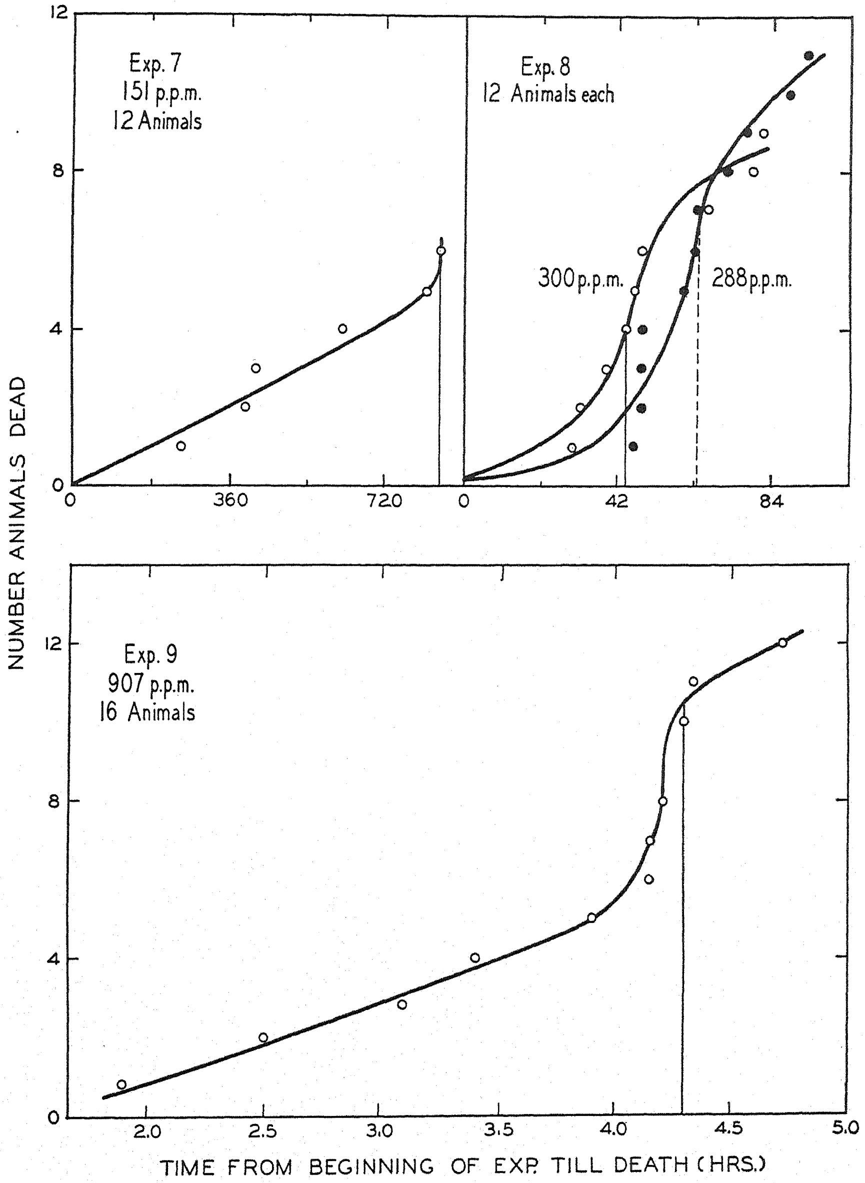

FIGURE 2. Mortality versus time toxicity curves for mouse experiments. The vertical lines to the curves indicate the times at which the SO_2-treatments were discontinued.

The problem of the effect of intermittent exposure to sulphur dioxide is of practical importance because industrial workers are exposed to the gas only about eight hours a day. Those experiments dealing with intermittent exposures are summarized in Tables III and IV. It will be noted that in addition to four-hour and eight-hour mono-daily exposures, some of the experiments included tests of the effects of one and two-hour exposures at five and four-hour intervals, respectively.

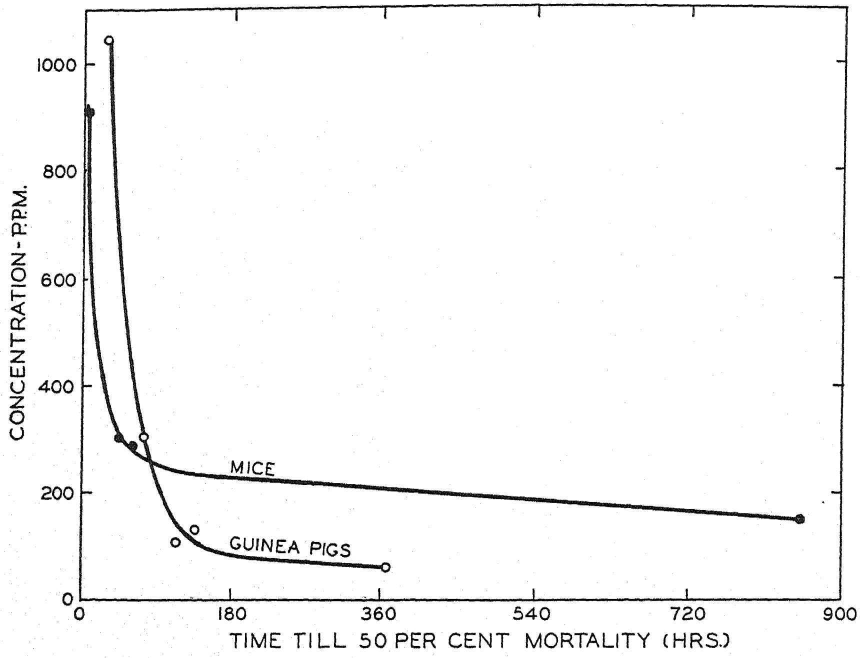

FIGURE 3. Time-till-50%-mortality versus concentration toxicity curves for mouse and guinea pig experiments.

Because it is a matter of common knowledge that exercise, with its resultant effect on volume of breathing, is an important factor in determining the rate of absorption of a gas, studies of the effects of exercise on susceptibility of guinea pigs were included in several experiments. The results of these experiments are summarized in Table V. As has been mentioned, no attempt was made to study the effect of exercise on mice.

The effects of previous sulphur dioxide treatments on susceptibility were investigated in several experiments, summarized in Table VI, in an effort to establish the extent of any cumulative or residual effects of gas treatment. In order to broaden further the basis for conclusions regarding the toxicity of sulphur dioxide, several experiments were planned to include

TABLE III

EFFECT OF INTERMITTENT EXPOSURE ON SUSCEPTIBILITY OF GUINEA PIGS TO SO_2

Exp. No.	Hours per day in SO_2	Av. SO_2 conc. (p.p.m.)	No. animals used	Total length of gas treatments (hrs.)	No. animals dead at end of treatment	Total No. animals dead 2 wks. after last treatment
5	24	25	10	1137	0	7*
	12	25	4	569	0	2*
	0	0	10	0	0	5**
6	24	112	8	113	4	7
	8	104	8	76	0	0
	4	105	8	40	0	0
	0	0	8	0	0	0
7	24	127	8	138	6†	8†
	24	131	8	154	4	5
	8	147	8	290	0	1
	4	148	8	136	0	0
	0	0	8	0	0	0
8	24	299	8	89	4	8
	8	281	8	153	3	4
	4	281	8	81	0	0
	0	0	8	0	0	0
9	24	1039	20	24	14	20
	12	1030	8	25	4	8

Note: Gas treatments were given in mono-daily increments except in the case of the 12-hour per day treatment in Exp. No. 9 where the animals were exposed 2 hours at a time at 2-hour intervals.

* Sacrified for autopsy.

** Four animals sacrificed for autopsy.

† Three animals sacrificed for autopsy.

studies of the effect of age of animals on susceptibility. The results of these experiments are summarized in Table VII.

The "theoretical" concentrations of the various experiments, as established by SO_2-flowmeter and air-manometer calibrations, together with temperature and humidity data, and dates of the experiments are given in Table VIII.

The various experimental gas treatments are discussed separately and in some detail under the experiment headings which follow.

Experiment No. 1 (63.7 p.p.m. SO_2 for 360 Hours)

The purpose of the first experiment was to furnish an opportunity to observe the rapid development of acute symptoms in order better to be able to detect the appearance of symptoms after less severe treatment. It was believed that 50 to 60 p.p.m. would be a sufficiently high concentration for this purpose. No white mice were available at the time, so only guinea pigs were used. Five mature guinea pigs, formerly used in insecti-

TABLE IV

EFFECT OF INTERMITTENT EXPOSURE ON SUSCEPTIBILITY OF MICE TO SO_2

Exp. No.	Hours per day in SO_2	Av. SO_2 conc. (p.p.m.)	No. of animals used	Total length of gas treatments (hrs.)	No. animals dead at end of treatment	Total No. animals dead 2 wks. after last treatment: No. sacrificed for autopsy	Total No. animals dead 2 wks. after last treatment: No. which died "naturally"
2	24	11.2	10	173	5*	0	5*
	0	0	10	0	0	0	0
	24	10.4	10	361	0	1	1
	12	10.7	10	180	0	1	4*
	0	0	10	0	0	1	4*
3	24	33.0	20	984	8*	2	8*
	12	32.3	7	492	2*	0	2*
	0	0	10	0	0	2	0
4	24	25.9	29	576	12*	0	12*
	12	26.2	15	276	3*	0	3*
	0	0	26	0	6*	0	6*
5	24	25.3	15	1137	0	3	0
	12	25.4	15	569	1*	3	5*
	0	0	15	0	4*	3	4*
6	24	109	12	238	0	0	2**
	8	104	12	76	0	0	3**
	4	105	12	40	0	0	0
	8†	106	8	76	0	0	0
	4††	106	8	40	1	0	1
	0	0	12	0	0	0	0
7	24	151	12	847	6	0	6
	8	147	12	265	0	0	0
	4	148	12	140	0	0	0
	8†	149	8	284	0	0	0
	4††	150	8	141	1	0	1
	0	0	12	0	0	0	2
8	24	300	12	45	4	2	7
	8	281	12	153	1	0	1
	4	281	12	80	0	0	0
	8†	295	8	165	2	0	2
	4††	298	8	84	0	0	0
	24	288	12	69	7	0	11
	0	0	12	0	1	0	1
9	24	907	16	4.3	9	0	13
	12	936	16	4.0	3	0	11

* Pathological findings show death due to epizootic enteritis.
** Died as result of accidental exposure to a very high concentration of SO_2.
† Exposed 2 hours at a time at 4-hour intervals.
†† Exposed 1 hour at a time at 5-hour intervals.

cide feeding experiments but now apparently normal, and five young guinea pigs recently purchased from a breeding company were treated with SO_2, and an equal number of similar guinea pigs were used for controls.

TABLE V

EFFECT OF EXERCISE ON SUSCEPTIBILITY OF GUINEA PIGS TO SO_2; ALL ANIMALS EXPOSED CONTINUOUSLY

Exp. No.	Av. SO_2 conc. (p.p.m.)	Length of gas treatment (hrs.)	Treatment	No. animals exposed to SO_2	No. animals dead at end of treatment	Length of exposure to SO_2 of animals that died in gas (hrs.)	Total No. animals dead 2 wks. after treatment
5	25.3	1137	Exercise	5	0	—	3*
			No exercise	5	0	—	4*
6	112	113	Exercise	4	2	54, 104	4
			No exercise	4	2	107, 113	3
7	127	138	Exercise	4	3	69, 96*, 138	4**
			No exercise	4	3	98*, 98*, 119	4†
7	131	154	Exercise	4	4	72, 120, 132, 146	4
			No exercise	4	0	—	1
8	299	89	Exercise	4	2	60, 69	4
			No exercise	4	2	73, 86	4
9	1039	24	Exercise	8	8	11.9, 11.9, 12.5, 12.5, 12.5, 12.6, 18.4, 19.2	8
			No exercise	12	6	14.6, 17.6, 17.6, 19.6, 21.0, 23.5	12

* Sacrificed for autopsy. ** One sacrificed for autopsy. † Two sacrificed for autopsy.

TABLE VI

EFFECT OF SO_2 PRETREATMENT ON SUSCEPTIBILITY OF GUINEA PIGS TO SO_2

Exp. No.	Av. SO_2 conc. (p.p.m.)	Length of gas treatment (hrs.)	SO_2 pretreatment	Time between pretreatment and present experiment (hrs.)	No. animals exposed to SO_2 in present experiment	No. animals dead at end of treatment	Total No. animals dead 2 wks. after treatment
3	33.0	984	63.7 p.p.m. for 360 hrs.	1497	2	0	1*
			None	—	12	1**	2*
4	25.9	576	33.0 p.p.m. for 984 hrs.	550	5	2**	2
			10.7 p.p.m. for 720 hrs.	2108	3	0	0
			None	—	14	5**	5
9	1039	24	300 p.p.m. for 153 hrs. (8 hrs. per day)	64	4	2	4
			None	—	16	12	16

* One sacrificed for autopsy.
** Pathological findings show death due to epizootic pneumonia.

The autometers were adjusted to discharge the absorbing solution every two minutes. Because of the large volumes of solution required, the autometer system was readjusted after 24 hours to discharge every 32 minutes and to sample from the inlet of the SO_2 cabinet only the last two minutes of each period. The average concentration for the run as obtained from 649 such readings was 63.7 p.p.m., the maximum recorded concentration was 73 p.p.m., the minimum 47 p.p.m.

TABLE VII

EFFECT OF AGE OF GUINEA PIGS ON SUSCEPTIBILITY TO SO_2

Exp. No.	Av. SO_2 conc. (p.p.m.)	Length of gas treatment (hrs.)	Age of animals (mos.)	No. animals exposed to to SO_2	No. animals dead at end of treatment	Length of exposure to SO_2 of animals that died in gas (hrs.)	Total No. animals dead 2 wks. after treatment
1	63.7	360	3–4	5	3	288, 353, 360	3
			12	2	1	219	1
			28	3	1	244	1
2	10.7	720	3–4	5	0	—	1*
			12	5	0	—	1*
3	33.0	984	4	5	0	—	0
			6	2	0	—	0
			12	5	1**	262	2*
			14	2	0	—	1*
4	25.9	576	3	5	1**	232	1
			6	5	2**	353, 460	2
			9	3	0	—	0
			12	5	4**	22, 278, 322, 329	4
			14	4	0	—	0
9	1039	23.6	6	4	1	17.6	4
	1030	25.4	6†	4	3	20.9, 21.1, 25.4	4
	1039	23.6	6††	4	2	14.6, 19.6	4
	1039	23.6	20	4	3	17.6, 21.0, 23.5	4
	1030	25.4	20†	4	1	14.4	4

Note: None of the animals listed in this experiment were exercised.
* One animal sacrificed for autopsy.
** Pathological findings show death due to epizootic pneumonia.
† Exposed alternately, 2-hour treatments at 2-hour intervals.
†† Pretreated: 8 hours per day of 300 p.p.m. (Exp. No. 8).

The guinea pigs were surprisingly resistant to the gas. They showed only slight symptoms of irritation when the gas was first turned on, and quickly became acclimated. After the first day they showed loss of appetite, on the fourth day one animal began to gasp, and by the eighth day all had developed a dyspnea which progressively became more pronounced until death or the end of the experiment. The first animal died on the ninth day and the fifth animal died on the fifteenth day. By the ninth day several guinea pigs had developed visibly distended abdomens. The five sur-

TABLE VIII

TEMPERATURE AND HUMIDITY DATA FOR THE EXPERIMENTAL FUMIGATIONS

Exp. No.	Theoret. SO_2 conc. (p.p.m.)	Date*	Temperature (°C.)						Relative humidity (%)					
			Fumigation cabinet			Control cabinet			Fumigation cabinet			Control cabinet		
			Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.
1	50–60	Oct. 1 to Oct. 16	66	82	58	69	83	60	55	70	22	58	80	30
2	10	Oct. 25 to Nov. 24	64	88	54	65	76	56	53	58	20	57	64	40
3	35	Dec. 16 to Jan. 26	66	85	51	67	93	59	50	68	20	53	60	18
4	25	Feb. 18 to Mar. 14	68	79	54	70	81	56	56	71	33	54	71	30
5	25	Apr. 4 to May 23	75	94	63	75	105	63	56	85	24	58	79	19
6	100	Nov. 16 to Nov. 26	67	95	61	73	95	62	57	71	12	62	73	18
7	150	Dec. 1 to Jan. 5	69	96	59	73	84	64	56	69	15	55	73	20
8	300	Jan. 9 to Jan. 29	78	107	62	74	97	53	52	69	16	50	66	13
9	1000	Feb. 1 to Feb. 3	78	96	73	76	85	71	44	53	34	49	68	41

* Exp. Nos. 1, 2, and 3 began in 1937; Exp. Nos. 4, 5, 6, and 7 in 1938; and Exp. Nos. 8 and 9 in 1939.

viving animals quickly recovered on removal from the gas, exhibiting no gross symptoms at the end of a week. Representative animals from this experiment are pictured in Figure 4.

The average weight of the mature guinea pigs, 747 grams, fell off 55 grams the first week and 60 grams the second. The young guinea pigs, averaging 321 grams, lost an average of 39 grams the first week and 9 grams the second. Both groups rapidly regained weight on removal from the gas. The control animals gained weight regularly throughout the experiment and exhibited no abnormal symptoms at any time.

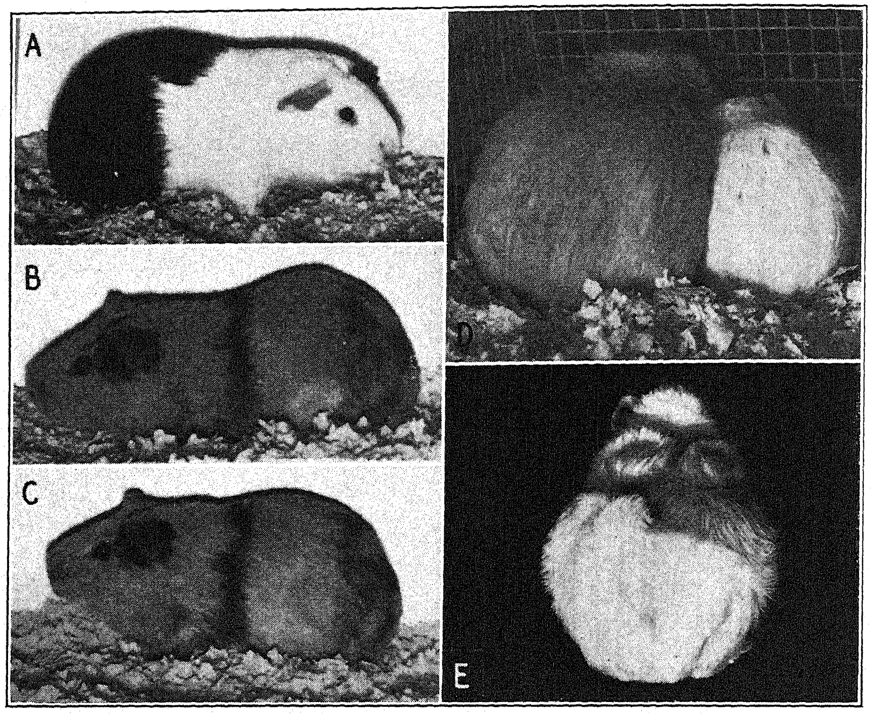

FIGURE 4. A. A one-year-old control guinea pig from Exp. No. 1 (63.7 p.p.m.) at end of experiment. B. A one-year-old animal from Exp. No. 1 after the first week of exposure. C. Same animal after second week of exposure. D. Guinea pig on left showing gross distention of abdomen after exposure to 127 p.p.m. SO_2 for 96 hours. E. Guinea pig showing gross distention of abdomen after exposure to 131 p.p.m. for 154 hours. (Photographed 14 hours after last exposure.)

The typical gross findings at autopsy are:

Lungs: Moderately edematous and congested, scattered small pleural hemorrhages.
Heart: In systole.
Stomach: Distended in about one-third of the animals.

Gall bladder: Distended.
Other organs: Slightly congested.

Experiment No. 2 (10.7 p.p.m. SO_2 for 720 Hours)

This experiment was designed to test the effect of a concentration representing the maximum which might be encountered for any length of time in a city atmosphere. The initial test animals were 20 young mature white mice, ten guinea pigs 12 months of age with an average weight of 635 grams, and ten guinea pigs three to four months old with an average weight of 335 grams. The animals were divided equally into SO_2 and control groups.

The autometers were adjusted to discharge the absorbing solution every 32 minutes, alternately sampling from inlet and outlet of the SO_2 cabinet for 28 minutes of each 32-minute period. The average sulphur dioxide concentrations for the different periods of exposure are given in the appropriate tables. The maximum concentration recorded for the whole run was 14.7 p.p.m. The minimum concentration of 6.4 p.p.m. occurred when the cabinet door was open to permit cleaning of the cabinet.

None of the guinea pigs exhibited any abnormal symptoms at any time during their 720 hours of exposure. The young SO_2-treated and the young control guinea pigs gained regularly each week during the gas treatment and during the three following weeks that their weights were noted. Both the old controls and the young SO_2 animals lost slightly the first week after they were transferred from the gas cabinet to the animal room, but they had regained more than their lost weight by the end of the third week of the post-gas period. Representative guinea pigs from this experiment are pictured in Figure 5.

The ten SO_2-treated mice suddenly became ill on the sixth day of exposure, four died on the seventh day, and one on the eighth. On the tenth day, two days after removal from the gas, the surviving mice seemed to have recovered. None of the control mice exhibited any abnormal symptoms and none of them died.

A second group of healthy, mature young mice was placed in the gas on the fifteenth day of the experiment. Of this group, ten were exposed to SO_2 continuously, ten were exposed from 11:30 A.M. to 11:30 P.M. each day, and ten were kept in the control cabinet. None of these mice exhibited any abnormal symptoms during the 15 days of gas treatment. In the two weeks following the gas treatment, eight mice died, but these post-gas deaths were about equally divided between the treated and the controls.

The typical gross findings at autopsy are:
Guinea pigs: No definite change.
Mice dying spontaneously: Lungs: Largely consolidated.
Intestine: Hemorrhagic.
Mice not apparently infected: No definite change.

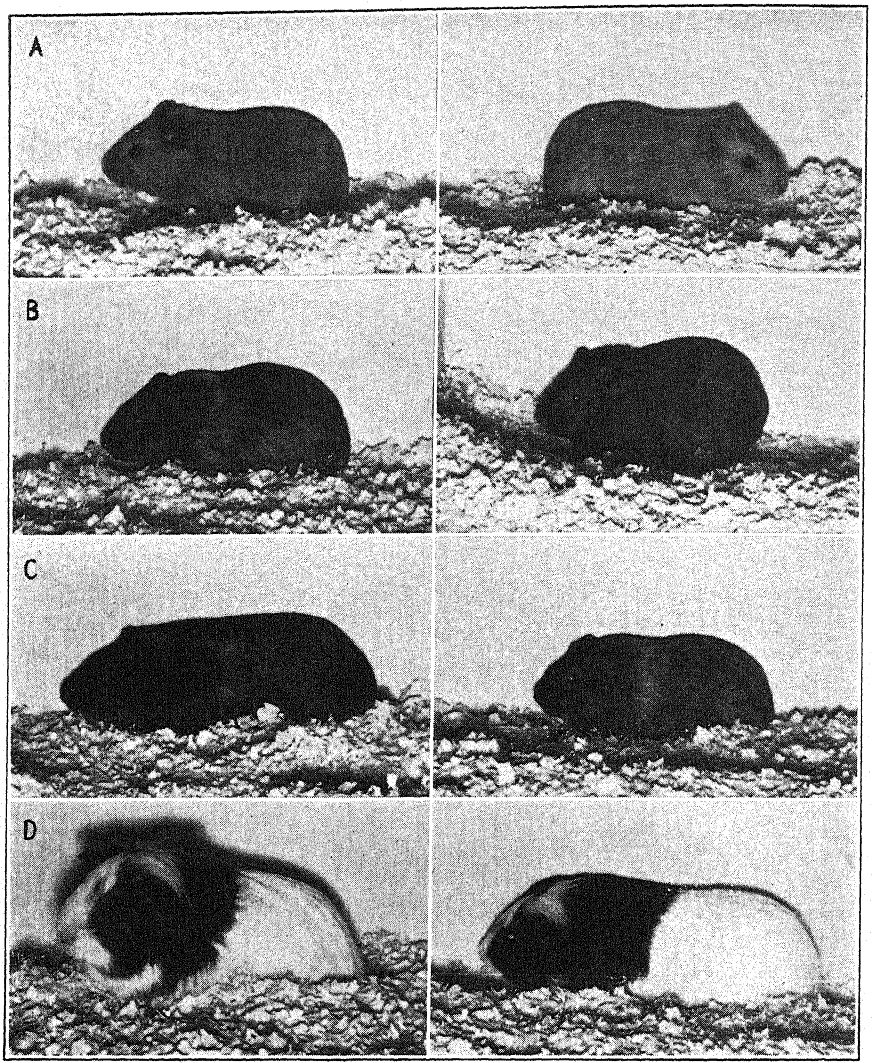

FIGURE 5. Guinea pigs used in Exp. No. 2 (10.7 p.p.m. SO_2). In each case two views of the same animal. Left, at beginning of experiment. Right, 720 hours later at end of experiment. A. Control, 3 to 4 months old. B. Treated, 3 to 4 months old. C. Control, one year old. D. Treated, one year old.

Experiment No. 3 (33.0 p.p.m. SO_2 for 984 Hours)

This experiment was designed to test the effect of a higher concentration of sulphur dioxide, than was used in Exp. No. 2, such as might be encountered for considerable periods in the course of certain industrial operations. The experimental animals were ten guinea pigs about 12 months old and ten guinea pigs about four months old, none of which had been used in any other experiments; four guinea pigs about 30 months old and four guinea pigs about six months old, half of which had been treated with 64 p.p.m. for 360 hours, the others of which had been controls in Exp. No. 1; and 37 young mature white mice, some of which had been in the first group of Exp. No. 2, others in the second group, and others in no previous experiment.

The autometers were adjusted as in the previous experiment. The average sulphur dioxide concentrations reported for the various periods of exposure are given in the appropriate tables. Beginning on the 25th day of the experiment and for a period lasting 36 hours, the animals were accidentally exposed to a concentration varying from 58 to 74 p.p.m. because the SO_2 orifice reading was thrown out of calibration by accidental clogging of the SO_2 feed line with rust and sulphur.

Of the 14 guinea pigs exposed to the gas only one, a 12-month-old, died. This guinea pig began to refuse food on the eighth day, and on the ninth day developed a dyspnea which progressively became more pronounced until death on the eleventh day. The other 13 guinea pigs slowly developed a slightly labored breathing which persisted to about the twelfth day after which the animals returned to normal until the twenty-fifth day when accidentally exposed to 58 to 74 p.p.m. At the end of the 31-hour high-concentration exposure, a pronounced dyspnea had developed, but on restoration of 30 to 40 p.p.m. the animals recovered, and they were apparently normal at the end of the experiment. The average weights of the treated and control guinea pigs dropped slightly during the first week of the experiment, but thereafter showed regular weekly gains. None of the control animals exhibited any abnormal symptoms. Representative guinea pigs from this experiment are pictured in Figure 6.

The experimental arrangement of the 37 mice and results of the gas treatment are given in Table IX. The various full-time mice died 21, 25, 26, 27, 27, 32, 32, and 37 days respectively, after the beginning of gas treatment. The half-time mice died on the eighteenth and twenty-fourth days. It is important to note that only one mouse died after the thirty-second day. All the SO_2 mice were sluggish after the high-concentration exposure, but those that survived had apparently recovered by the end of the experiment. None of the control mice exhibited any abnormal symptoms.

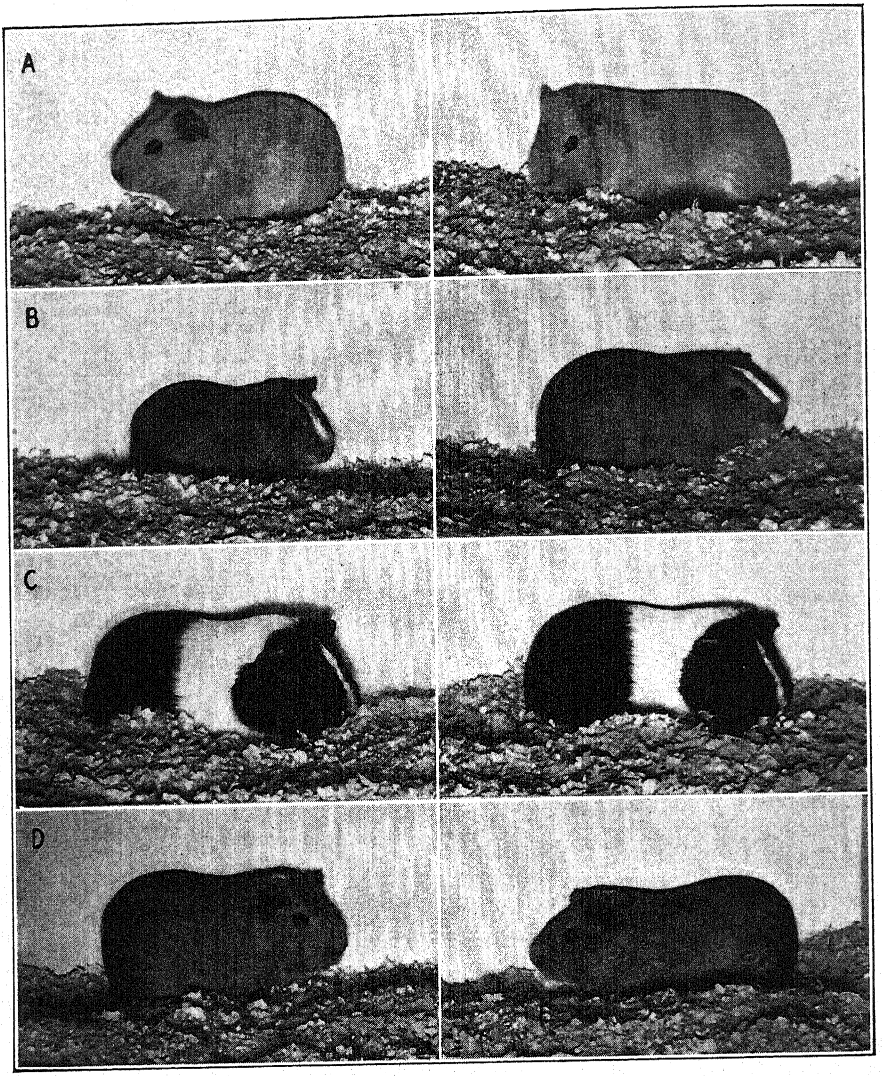

FIGURE 6. Guinea pigs used in Exp. No. 3 (33.0 p.p.m. SO_2). In each case two views of the same animal. Left, at beginning of experiment. Right, 984 hours later at end of experiment. A. Control, 3 to 4 months old. B. Treated, 3 to 4 months old. C. Control, about one year old. D. Treated, about one year old.

TABLE IX

ARRANGEMENT OF MICE, EXP. NO. 3

Hours per day in SO_2	Group No.	No. mice in cage	Previous SO_2 treatment	No. mice dead at end of exp.
24	2	7	10.4 p.p.m. for 361 hrs.	1
	2	7	None-controls for Exp. 2	3
	1	3	10.8 p.p.m. for 173 hrs.	1
	1	3	None-controls for Exp. 2	3
12	2	7	None-controls for Exp. 2	2
0	2	6	10.4 p.p.m. for 180 hrs.	0
	1	2	10.8 p.p.m. for 173 hrs.	0
	1	2	None-controls for Exp. 2	0

The typical gross findings at autopsy are:

Lungs: Slightly edematous, occasional small pleural hemorrhages.

Gall bladder: Distended.

It is possible that the nasal discharge beginning on the eighth day and followed by temporary dyspnea which coincided with the fatal pneumonia of one animal was caused partly by infection.

Experiment No. 4 (25.9 p.p.m. SO_2 for 576 Hours)

This experiment was designed as a further test of the toxicity of sulphur dioxide in concentrations frequently encountered for considerable periods in certain industries. The experimental animals were ten guinea pigs about 12 months of age, and ten guinea pigs about three months of age, none of which had been used in any other experiment; 23 guinea pigs formerly used in Exp. No. 2 or 3, 48 white mice, none of which had been used in any other experiment; and 22 mice formerly used in Exp. No. 3.

The age and pretreatments accorded the 23 guinea pigs can be determined largely from Tables VI and VII. The 22 mice were divided into two equal groups in such a manner as to randomize all previous treatments and group numbers. The 48 mice were divided into 15 controls, 15 half-time mice exposed from 11:30 P.M. to 11:30 A.M. each day, and 18 full-time animals.

The autometers were adjusted as in Exp. No. 2. The average sulphur dioxide concentrations (mean of inlet and outlet readings) for the various exposure periods are given in the appropriate tables. The maximum concentration recorded for the whole gas treatment was 37 p.p.m.; a minimum of 3 p.p.m. was recorded for a 32-minute period when the gas and air flow were discontinued while the cabinet was being cleaned.

The new group of guinea pigs and mice were, unfortunately, not quarantined prior to use but were placed in the experiment immediately on receipt from the breeders. Two guinea pigs, used in the SO_2 group, were dyspneic on arrival. One of the two was very weak and died the first day. Four more of this group died on the tenth, twelfth, fourteenth, and fifteenth days, respectively, and two of the new-group control guinea pigs died on the tenth and thirteenth days, respectively. One of the old SO_2-group died on the fifteenth day, another on the twentieth day, and an old-group control guinea pig died on the twenty-first day of gas treatment. All those guinea pigs that died lost weight rapidly the week before death. The average weight of the surviving guinea pigs, both treated and controls, stayed about the same throughout the gas treatment.

Almost 75 per cent of the mice became ill very suddenly; and seven full-time mice, two alternates, and five control mice died during the gas treatment. Several mice died during the next few days, but by the seventeenth day all the survivors seemed to have recovered. On the twenty-third day, however, three more full-time mice died. In all, 12 SO_2-mice, three alternates, and six controls died during the gas treatment. The deaths were divided about equally between pretreated and non-pretreated mice.

The typical gross findings at autopsy are:

Lungs: Largely consolidated.

Other organs: Congested.

Experiment No. 5 (25.3 p.p.m. SO_2 for 1137 Hours)

This experiment was designed to repeat Exp. No. 4 using healthy, uninfected animals. All the animals on hand were destroyed; the animal quarters, food bins, drinking devices, etc. were sterilized in the autoclave, the treatment cabinets were washed out with Lysol solution followed by hosing with water, and a new group of 24 guinea pigs and 45 white mice were quarantined two weeks before use in the experiment.

The autometers were adjusted as in Exp. No. 2. The average sulphur dioxide concentrations (mean of inlet and outlet readings) for the various exposure periods are given in the appropriate tables. The maximum concentration recorded for the whole gas treatment was 39 p.p.m.; a minimum of 7 p.p.m. was recorded when the gas and air flow were discontinued while cleaning the cabinet.

All the guinea pigs were three to four months old at the beginning of the experiment, and they were divided by chance into four groups of five and one group of four. The four-membered group was exposed to SO_2 from 11:30 P.M. to 11:30 A.M. each day, two of the five-membered groups were exposed continuously to the gas, and two served as controls. The alternate guinea pigs, one group of controls, and one group of full-time exposure were exercised two hours daily except Sunday.

There were no guinea pig fatalities during the course of the treatment. Throughout the run, however, the SO_2-guinea pigs were somewhat lethargic compared with the controls, and two exercised and two non-exercised

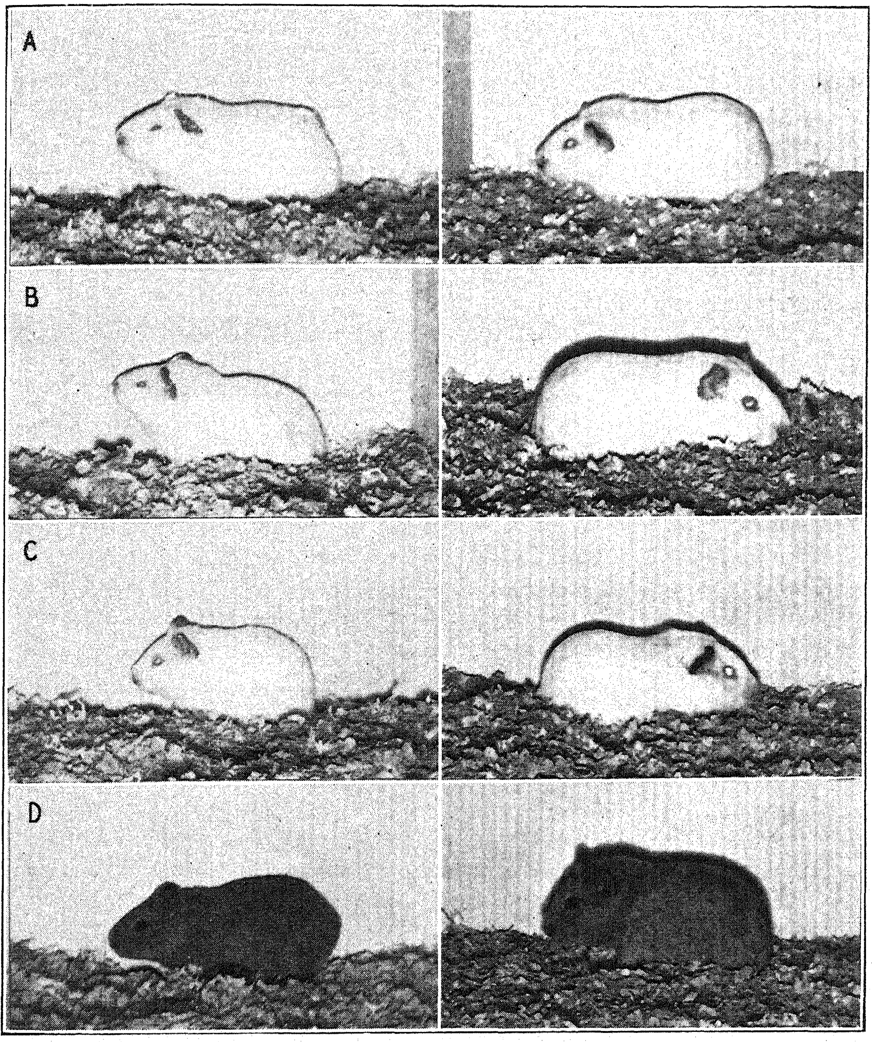

FIGURE 7. Guinea pigs used in Exp. No. 5 (25.3 p.p.m. SO_2). In each case two views of the same animal. Left, at beginning of experiment. Right, 1137 hours later at end of experiment. A. Control, not exercised. B. Treated, not exercised. C. Control, exercised. D. Treated, exercised.

SO_2-guinea pigs developed a slight dyspnea for short periods but recovered during the course of the run. All the groups gained in weight regularly throughout the gas treatment except the exercised SO_2-guinea pigs which showed a drop in average weight from 369 to 359 grams during the sixth week of treatment. All the guinea pigs that were not sacrificed for autopsy gained weight over a three-week post-gas observational period except the one remaining SO_2-exercised guinea pig which dropped from 361 to 335 grams. Representative guinea pigs from this experiment are pictured in Figure 7.

The 45 mice were divided by chance into three groups of 15. One group was exposed to the SO_2 from 11:30 P.M. to 11:30 A.M. daily, one group was exposed continuously, and the third group served as controls. One of the control mice died the day after the experiment began, and a total of four controls died before the end of the experiment. None of the full-time or alternate mice died during the gas treatment. Three of each group were sacrificed for autopsy at the end of the gas treatment; and five alternates, but no full-time or control mice, died during a three-week post-gas period. All living mice were weighed on the seventh and forty-eighth days of the experiment, and each group showed a gain in average weight over the period.

The typical gross findings at autopsy are:

Lungs: Very slightly to moderately distended, occasional small pleural hemorrhages.

Gall bladder: Distended.

Experiment No. 6 (109 p.p.m. SO_2 for 238 Hours)

Exp. Nos. 6, 7, 8, and 9 were designed to test the toxicity of relatively high concentrations of sulphur dioxide and to study the development of certain interesting pathological symptoms. Since these four high-concentration experiments were very similar in design and in procedure, Exp. No. 6 will be described in some detail to serve as a reference and illustration.

The autometers were adjusted to discharge the absorbing solution every 32 minutes and to sample from the inlet and the outlet of the SO_2 cabinet four minutes of every 32. During the first two-minute sampling period air was drawn from the autometer cabinet to furnish a conductivity datum line, during the next two sampling periods air was drawn from the inlet and outlet of the SO_2 cabinet, and during the remaining 13 sampling intervals, in the 32-minute run, the air was drawn from the greenhouse. This adjustment was used in all the remaining experiments. In Exp. Nos. 7 and 8 the volume of absorbing solution was increased from the usual 100 ml. to 175 ml.

The animals used in Exp. Nos. 6, 7, 8, and 9 were all taken from a uniform group of 100 guinea pigs and 200 mice. The animals were quarantined and their weights determined for a month before they were used in Exp. No. 6. All cages, food bins, etc. were sterilized in an autoclave before contact with the animals. The guinea pigs were about four months old weighing an average of 343 grams; the mice were young mature, and active, and all the animals were apparently in good physical condition at the beginning of Exp. No. 6.

Thirty-two guinea pigs were selected at random from the group of 100, and divided into eight sub-groups of four so that the average weights of the sub-groups were as nearly the same as possible. Two of these sub-groups were exposed to the gas continuously, two were exposed eight hours per day, two were exposed four hours per day, and two sub-groups were used as controls. One of each pair was exercised two hours per day. The exercising of each group except the controls was done in the SO_2 cabinet. The alternate exposures were given in mono-daily increments.

Sixty-four mice were selected at random from the group of 200, and divided into four sub-groups of 12 and two sub-groups of eight. The four 12-membered groups were treated with SO_2 in mono-daily increments of 24, 8, 4, and 0 hours, respectively. One eight-membered group was exposed one hour at a time at five-hour intervals, the other was exposed to the gas two hours at a time at four-hour intervals.

The average SO_2 concentrations for the various exposure periods are given in the appropriate tables. The maximum concentration recorded up until the end of the run was 137 p.p.m., the minimum was 62 p.p.m. On the tenth day the experiment was discontinued because of the accidental development of a very high exposure. This high concentration prevailed for 15 minutes before being discovered, and by the time it had been corrected, two mice had died and all the mice were in great distress, their fur had a wet and mottled appearance and they were gasping spasmodically. Within two hours after this exposure, four more mice died but those that survived had apparently recovered completely within 48 hours. The mouse fatalities were distributed among the several groups irrespective of previous gas treatment.

One of the four guinea pigs in the exerciser at the time of the accident developed a pronounced dyspnea, but none of the others, or any of the four not being exercised, showed any symptoms other than a marked lethargy. None of the guinea pigs exhibited any adverse symptoms 48 hours later. Unfortunately, the conductivity reading went so far off the chart that no estimation of the peak concentration can be made.

The accident occurred before any of the groups of mice or of alternate guinea pigs had reached the 50 per cent mortality, but half of the full-time guinea pigs had died by the fifth day.

When the gas treatment began the guinea pigs exhibited no unusual symptoms, but within 12 hours they were noticeably lethargic as compared with the controls. Within 23 hours the nasal mucosa showed signs of irritation, and within 45 hours all the continuously-exposed guinea pigs had developed a pronounced dyspnea. As the experiment continued the dyspnea became more pronounced, the nostrils became clogged with bloody mucus, a glassy opaque film developed over their eyes, the animals became so weak they were unable to stand, and finally an apparent paralysis of the hind quarters set in several hours before death. The first guinea pig died 54 hours after the experiment began, and the 50 per cent point was reached in 113 hours. Three more guinea pigs died within 24 hours after removal from the gas. The guinea pigs exposed eight hours per day developed a slightly labored breathing after about 40 hours of actual gas treatment and were somewhat lethargic when the experiment ended after 76 hours of gassing. The guinea pigs exposed four hours per day showed no abnormal symptoms after 40 hours of actual exposure. Each of the exposure groups, except the full-time guinea pigs, gained weight during the gas treatment.

The mice, on first exposure to the gas, were visibly disturbed, sniffing and rubbing their noses, but after about an hour they had apparently become accustomed to the gas and showed no further evidence of annoyance. After 24 hours of treatment, the full-time mice were inactive and huddled together in groups considerably more than the controls or part-time mice. After 48 hours they seemed to have difficulty in breathing and after 130 hours of gas they had developed a pronounced dyspnea, and showed dried secretions at the nostrils. One four-hour per day mouse died after 34 hours of gas treatment but there were no other fatalities until the very high concentration exposure at the end of the run. Practically all animals that died or were killed showed distended abdomens.

It is of interest to note that the mice were more resistant to the lower concentration than were the guinea pigs, but more susceptible to the higher concentration.

The fact that other experimenters have always found mice to be the more susceptible suggested that the greater resistance at the low concentration may have been merely the result of extreme naturally-occurring variation, but subsequent experiments clearly support the finding of differences in slope of the two toxicity curves.

The typical gross findings at autopsy are:

Cornea: Opaque.

Trachea: Not visibly reddened.

Lungs: Moderately edematous, pale with occasional small hemorrhages of the pleura.

Heart: In systole.

Stomach: Distended.
Gall bladder: Distended in about half the animals.
Other organs: Slightly congested.
In one guinea pig the liver was ruptured and in two the stomach.

Experiment No. 7 (151 p.p.m. SO_2 for 847 Hours)

The design of this experiment and choice of material was the same as that of Exp. No. 6. The concentration was set at 150 p.p.m., but actually ran about 130 p.p.m. for the first few days due to a feed line obstruction. The maximum concentration recorded was 180 p.p.m.; the concentration dropped below 100 p.p.m. several times while the cabinet was being cleaned, but only for short periods.

The guinea pigs suffered some slight irritation on first exposure to the gas, as evidenced by rubbing of noses with fore paws, but seemed to have become accustomed to the gas within five or ten minutes. After 16 hours of treatment the nasal mucosa of the full-time guinea pigs showed a reddening and within 23 hours the nostrils were completely clogged with a bloody mucus. Within 35 hours the guinea pigs developed a slight dyspnea which became pronounced within 48 hours. As the gas treatment continued the dyspnea became more pronounced, distention of the abdomen became apparent, opaque films covered the eyes, systemic weakness progressed, and hind-quarter paralysis set in. The first guinea pig died after 69 hours of gas treatment. In order to obtain fresh tissue for pathological study, three guinea pigs most severely affected were sacrificed for autopsy after 98 hours of treatment. The third natural death, 60 per cent point, occurred 138 hours after the experiment began. The two surviving guinea pigs died 19 and 77 hours after removal from the gas.

The full-time treatment was repeated using eight newly-selected guinea pigs. Again the animals exhibited some slight distress on first exposure, but seemed habituated to the gas within 15 minutes, and the symptoms continued to develop much as before. Several times it was noted that dyspnea became more pronounced after feeding. All the guinea pigs had grossly distended abdomens in the latter stages before death. The first animal died after 72 hours of gas treatment and the 50 per cent point was reached in 146 hours, but the surviving animals were not removed from the gas until after 154 hours of treatment. One guinea pig died 24 hours after removal from the gas and another died 16 days later. Abdominal distention in representative surviving guinea pigs is pictured in Figure 4, D and E.

The guinea pigs exposed intermittently showed signs of slight irritation every time they were moved into the gas. After 14 days of treatment the eight-hour guinea pigs were somewhat lethargic while in the gas but were active when in the control cabinet. On the twentieth day some irritation of the nasal passages was evident, on the twenty-ninth day abdominal distention was apparent in several guinea pigs, and a slight dyspnea had developed which became pronounced in the case of one guinea pig by the thirty-fourth day. No further symptoms developed during the 35 days of the experiment, 290 hours of actual gas treatment, and the animals completely recovered during a two-week, post-gas observational period. The four-hour guinea pigs developed no abnormal symptoms, other than a slight lethargy, during their 136 hours of actual gas treatment. Every guinea pig showed a gain in weight during the treatment except the full-time animals and the one eight-hour guinea pig that developed pronounced dyspnea. The full-time guinea pigs lost an average of 67 grams the first week, while the eight-hour animal lost 135 grams during the last three weeks of gas treatment.

To test the possibility of indirect toxic effects resulting from eating food exposed to SO_2, eight untreated guinea pigs in the animal room were fed carrots and cabbage which had been exposed to 150 p.p.m. for periods of one to eight days. The guinea pigs were fed 70 grams of SO_2-treated cabbage or carrots each day for 34 consecutive days with the exception of seven days when sufficient treated food was not available and they were fed non-treated cabbage or carrots. The animals exhibited no abnormal symptoms throughout the experiment and showed a regular weekly gain in weight.

The mice showed some irritation on first exposure to the gas but quickly became acclimated. After 40 hours, lethargy and a slight dyspnea were apparent in the full-time mice. These symptoms became more pronounced, a nasal irritation and abdominal distention became apparent, and a glassy opaque film spread over the cornea of the eye as the experiment continued. The first mouse died after 249 hours of gas treatment, the 50 per cent point was reached in 847 hours. The mice exposed eight hours per day had developed a slight dyspnea by the eighteenth day, but there were no fatalities during the experiment. The four-hour mice showed no symptoms other than a lethargy while in the gas. One mouse died on the third day after 12 hours of treatment, but pathological examination showed that death was not due to sulphur dioxide.

The typical gross findings at autopsy are:

Cornea: Opaque.
Trachea: Not visibly reddened.
Lungs: Moderately edematous, slightly to moderately congested; scattered pin point hemorrhages in the pleura and cut surfaces.
Heart: In systole.
Stomach: Distended.
Gall bladder: Distended (in guinea pigs only).
Other organs: Slightly congested.

A few animals show multiple ulcers of the gastric mucosa from pin point to 0.3 cm. diameter. All ulcers of the larger size were accompanied by circular subjacent hemorrhages in the wall.

Experiment No. 8 (296 p.p.m. SO_2 for 484 Hours)

The design of this experiment and choice of material was the same as that of Exp. No. 6. The maximum concentration recorded was 356 p.p.m. The concentration dropped below 250 p.p.m. six times but only for short periods while the cabinet was being cleaned or animals moved.

The guinea pigs showed some slight irritation on first exposure to the SO_2 but were not sufficiently affected to stop eating. They seemed habituated to the gas after five to ten minutes. Evidences of dyspnea and of nasal irritation became apparent on the second day of exposure of the full-time guinea pigs. Abdominal distention and drying out of the cornea were clearly evident on the third day, the first death occurred after 60 hours of treatment, and 50 per cent mortality was reached in 86 hours. The surviving animals were removed from the gas after 89 hours of treatment, but all of them died within 14 hours. In almost every instance paralysis of the hind quarters was noted one to two hours before death.

The guinea pigs exposed eight hours per day exhibited a pronounced dyspnea after 48 hours of actual gassing, gross irritation of the nasal mucosa and abdominal distention became apparent after 60 hours, and the first death occurred after 63 hours of actual SO_2 treatment. The third death occurred after 127 hours of gassing, and the fourth animal died 12 hours after his eight-hour treatment, having been exposed to SO_2 for 153 hours. None of the four survivors died during the post-gas observational period.

The four-hour per day guinea pigs showed some evidence of abdominal distention and dyspnea after 45 hours of gassing, but no fatalities occurred during the 20 days of the experiment, or 81 hours of actual gas treatment.

The guinea pigs were weighed twice weekly. All the animals including controls lost weight the first three days of the experiment but the controls and four-hour guinea pigs regained their losses within the next week. The eight-hour animals lost weight throughout the treatment, but began to regain after removal from the gas.

As in Exp. No. 7, eight untreated guinea pigs in the animal room were fed with SO_2-treated food to test the possibility of indirect toxic effect. The animals were fed 70 grams of SO_2-treated cabbage or carrots each day for 18 consecutive days with the exception of one day when they were fed untreated carrots. The treated food had been exposed to 300 p.p.m. for periods of two to eight days. The animals developed no abnormal symptoms, but their average weight dropped from 451 to 433 grams during the 18 days while the average weight of control guinea pigs fed no treated food rose from 459 to 462 grams. The loss in weight may have been due to the dehydration of the food exposed to SO_2.

The mice showed some evidence of irritation whenever first exposed to the gas, but as in the other experiments quickly became habituated. Dyspnea became evident in those continuously exposed after ten hours of treatment, the first mouse died in 28 hours, the fourth of 12 in 39 hours. Two very sick mice were sacrificed to obtain fresh tissue for pathological study, and the mice were removed from the gas after 45 hours. Three of the six surviving mice died during the two-week observational period.

Distention of the abdomen was difficult to detect in the early stages because of the tendency of the mice to huddle together, but all the mice suffered from considerable distention in the latter stages of treatment.

The full-time exposure was repeated with a new group of 12, and symptoms developed much the same as outlined above. The first mouse died in 46 hours, the fourth in 48 hours, and the sixth in 63 hours. One more mouse died before the group was removed from the gas after 69 hours, and four more mice died during the following 24 hours.

The eight-hour mice showed symptoms of dyspnea after 13 hours of exposure, one mouse of the mono-daily exposure group died after 24 hours of exposure, one of the eight-hour exposure group died after 24 hours of exposure, two of the two-hour exposure group died after 87 and 111 hours, respectively, but no further deaths occurred during the remainder of the treatment.

The four-hour mice developed slight evidences of dyspnea but no fatalities occurred during their 80 to 84 hours of gas treatment.

It should be noted that in this experiment the mice were more susceptible than the guinea pigs when exposed continuously, but that they were more resistant to the intermittent exposures.

Typical gross findings at autopsy are:

Guinea pigs:

Cornea: Opaque.
Lungs: Hemorrhagic and edematous, less distended than at lower concentrations.
Heart: Right ventricle dilated.
Stomach: Distended, multiple ulcers and hemorrhages.
Gall bladder: Distended.
Other organs: Moderately congested.

Alternate animals show less hemorrhage of the lungs and fewer ulcers of the stomach. Also the eight-hour unexercised group did not show distention of the gall bladder.

Mice:

Cornea: Opaque.
Lungs: Much hemorrhage and edema.
Heart: Right ventricle dilated.

Stomach: Much distended; many ulcers and hemorrhages.
Gall bladder: Not distended.
Other organs: Moderately congested.

In the alternate animals the stomach was less frequently affected.

Experiment No. 9 (1014 p.p.m. SO_2 for 49.4 Hours)

The history and experimental arrangement of the 28 guinea pigs and 32 mice used in this experiment are summarized in Tables X and XI together with mortality figures. The 20-month-old guinea pigs were formerly used in Exp. No. 5.

TABLE X

GUINEA PIG DATA, EXP. NO. 9

Age (mos.)	Treatment	Pretreatment	No. animals used	Length of exposure to SO_2 of animals that died in gas (hrs.)	Post-gas deaths	
					Total gas treatment (hrs.)	Time after last treatment until death (hrs.)
20	Exposed continuously, exercised	None	4	11.9; 12.5; 12.6; 19.2	—	—
	Exposed continuously, no exercise	None	4	17.6; 21.0; 23.5	23.6	41.8
	Exposed intermittently, no exercise	None	4	14.4	25.4	4.8 9.5 90.4
6	Exposed continuously, exercised	None	4	11.9; 12.5; 12.5; 18.4	—	—
	Exposed continuously, no exercise	None	4	17.6	23.6	1.1 17.5 43.8
		8 hours per day of 300 p.p.m. (Exp. 8)	4	14.6; 19.6	23.6	30.8 35.7
	Exposed intermittently, no exercise	None	4	20.9; 21.1; 25.4	25.4	17.9

Note: The guinea pigs exposed intermittently were given 2-hour gas treatments at 2-hour intervals. The exercised aimals were exercised 2 hours at a time at 2-hour intervals.

The average concentration for the time when the continuously exposed guinea pigs were in the gas was 1039 p.p.m.; the average concentration for the intermittently exposed guinea pigs was 1030 p.p.m.

The autometers were adjusted to discharge absorbing solution every two minutes and the inlet and outlet of the SO_2 cabinet was sampled for 28 minutes of every 32. Because of the high concentration the volume of air sampled had to be very small to keep the conductivity reading on the recorder chart; and small fluctuations in the air sampled caused large differences in individual recorder readings although they were without appreciable effect on the average of many readings. Because of this fact the range in recorder readings from 650 to 1400 p.p.m. represents many times the concentration fluctuation which actually occurred. The recorder range could have been changed, but it was felt that the resultant advantage for this one experiment would not warrant the necessary effort and delay.

TABLE XI

MOUSE DATA, EXP. NO. 9

Treatment	Pretreatment	No. animals used	Length of exposure to SO_2 of animals that died in gas (hrs.)	Post-gas deaths	
				Total gas treatment (hrs.)	Time after last treatment until death (hrs.)
Exposed continuously	None	8	2.5; 3.1; 4.1; 4.3	4.3	0.1; 0.4; 152.4
	4 hrs. per day of 300 p.p.m. (Exp. 8)	8	1.9; 3.4; 3.9; 4.1; 4.2	4.3	100.1
Exposed intermittently	None	8	—	4.0	0.1; 1.4; 16.2; 80.6; 148.9; 201.3
	4 hrs. per day of 300 p.p.m. (Exp. 8)	8	1.9; 3.6; 2.0*	4.0	0.1; 201.3

Note: The mice exposed intermittently were given 2-hour gas treatments at 2-hour intervals. There was an elapsed time of 64.2 hours between the pretreatment and beginning of the present experiment. The average concentration for the time when the continuously exposed mice were in the gas was 907 p.p.m.; the average concentration for the intermittently exposed mice was 936 p.p.m.

* Died during first non-gas interval 3 minutes after last exposure to gas.

On first exposure to the gas the guinea pigs suffered considerable irritation as evidenced by lachrymation, restlessness, coughing, sneezing, and rubbing of noses, but their distress was considerably less than had been anticipated from experiments described in the literature. Dyspnea and abdominal distention were apparent in a few hours, and after 12 hours of gas treatment a pronounced cloudiness of the cornea had developed. Motion pictures of a pair of guinea pigs were taken at intervals during the first six hours of exposure. The two-hour rest period between two-hour exposures was not sufficient to permit recovery and the alternate guinea pigs were affected as much as the continuously exposed guinea pigs by equal periods of gas treatment. Age and pretreatment had no significant effect on susceptibility, but the exercised guinea pigs were much more susceptible than the non-exercised.

The hours of gas treatment till 50 per cent mortality (both ages) of the full-time exercised, full-time non-exercised, full-time pretreated non-exercised, and alternate non-exercised were 12.5, 23.5, 19.6, and 25.4 hours, respectively. Although the surviving animals in each group were removed from the gas shortly after the 50 per cent point had been reached, all the animals died within 91 hours after treatment.

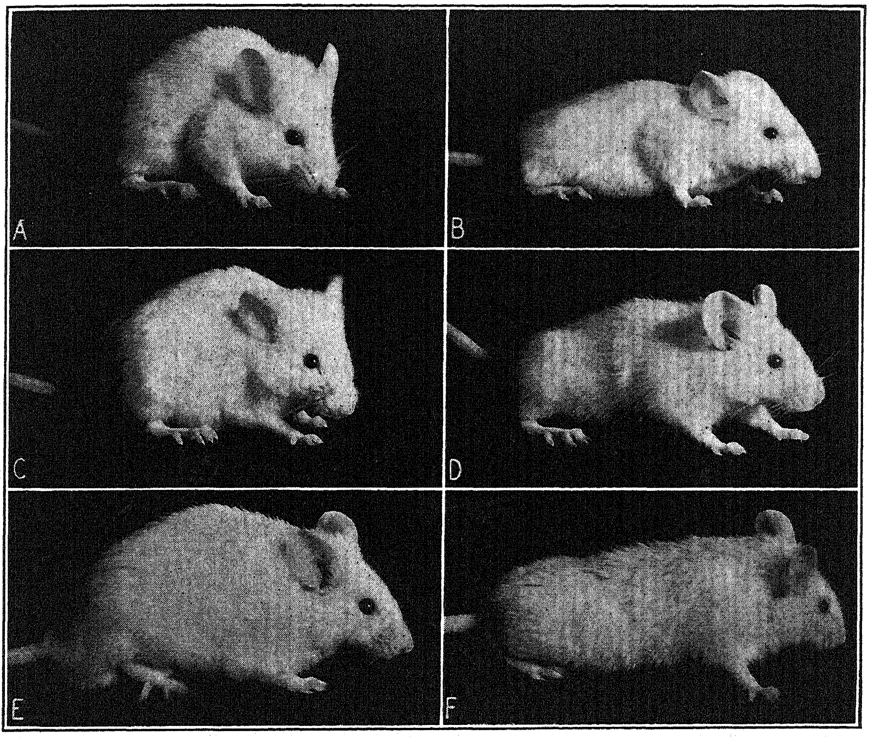

FIGURE 8. Mice exposed to sulphur dioxide. A. After exposure to 10.7 p.p.m. for 7 wks. B and C. After exposure to 33 p.p.m. for 6 wks. C. Pretreated with 10.7 p.p.m. for 7 days. D. After alternate exposure (12 hours) to 33 p.p.m. for 6 weeks. E. After exposure to 938 p.p.m. for 4 hours. Had been previously exposed to 281 p.p.m. for 3 days. (Photographed 40 days after exposure.) F. After exposure to 938 p.p.m. for 4 hours. (Photographed 40 days after exposure.) (*Note:* All mice apparently normal when photographed.)

The white mice were much more susceptible than the guinea pigs to this high concentration. They were very restless on first exposure to the gas, but were noticeably inactive and suffered some difficulty in breathing after ten minutes. After 20 minutes dyspnea was very pronounced and the first mouse died in 111 minutes. As with the guinea pigs, neither pretreatment nor intermittent exposure effected susceptibility. The fourth, fifth, and sixth alternate-group deaths occurred while the group was in the control cabinet so these mice were given no further SO_2 treatment because of the desire to have some of the mice survive the treatment in order to observe the development of pathological symptoms. Five alternate mice and three of those exposed continuously lived through a six-week, post-gas observational period. Two mice of this group are shown in Figure 8 together with mice that had been treated with lower concentrations of the gas.

The typical gross findings at autopsy are:

Guinea pigs:
  Trachea: Mucosa swollen, sometimes reddened.
  Lungs: Moderately to severely edematous and hemorrhagic, never extremely so.
  Heart: Right ventricle dilated.
  Stomach: Distended, multiple usually small ulcers and hemorrhages
  Gall bladder: Distended.
  Other organs: Congested.

Mice:
  Trachea: Fluid blood present.
  Lungs: Extremely edematous and hemorrhagic.
  Heart: Right ventricle dilated.
  Stomach: Distended, rare pin point hemorrhages.
  Gall bladder: Not distended.
  Other organs: Congested.

INVERTEBRATES

Sulphur dioxide, usually obtained by burning sulphur, has long been used against household insects, but the concentrations used have not been known. Recently, Gough (6) reported results on the relative resistance of the different stages of the bedbug (*Cimex lectularius* L.) to sulphur dioxide. He exposed eggs from 0 to 8 days old, nymphs of all stages, and adults to known concentrations of sulphur dioxide for two and one-half hours at a temperature of 23° C., and a relative humidity of 60 per cent. The concentrations ranged from 4.2 mg./l. (1600 p.p.m.) to 16.7 mg./l. (6400 p.p.m.). Newly laid eggs, he states, were considerably more resistant than either the adult or nymph, and the resistance greatly decreased as the time of hatching approached.

Grasshoppers

Nymphs[2] of *Melanoplus differentialis* (Thomas), chiefly of the third and fourth instars, were confined in wire cages placed in the SO_2 cabinet.

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[2] Hatched from eggs furnished through the courtesy of G. A. Dean and Donald A. Wilbur of Manhattan, Kansas.

One hundred nymphs were exposed to each concentration (25 p.p.m., 76 p.p.m., and 107 p.p.m.), and a similar number were used as controls in each experiment. The distribution of the nymphs in the cages was the same in both the SO_2 cabinet and control cabinet for each concentration stated above. Thirty nymphs caged in groups of five were exposed to a concentration of 938 p.p.m., while an equal number of nymphs caged in a similar manner were used as controls. The experiments were planned to determine the 50 per cent mortality point for each concentration, which was attained for concentrations of 107 p.p.m. and 938 p.p.m. The resistance of the insect was found to be so great to the lower concentrations (25 p.p.m. and 76 p.p.m.) that it was found impractical to prolong the exposures for a sufficient length of time to reach the 50 per cent point.

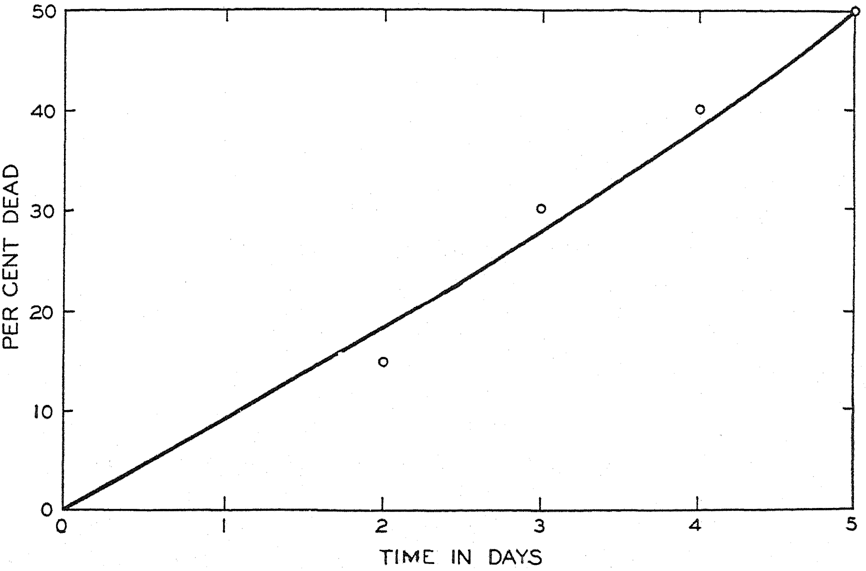

FIGURE 9. Mortality versus time toxicity curve for grasshopper nymphs (*Melanoplus differentialis*) exposed to 107 p.p.m. The experiment was discontinued at the 50 per cent point.

The results are shown graphically in Figure 9. It will be noted that at a concentration of 107 p.p.m. it required five days' continuous exposure to kill 50 per cent of the nymphs. At 76 p.p.m. 25 per cent were dead in 12 days, while at a concentration of 25 p.p.m., even though the exposure was for 21 days, the kill was only 30 per cent. The mortality in the controls ranged from 1 per cent to 10 per cent.

The signs in living exposed grasshoppers to 938 p.p.m. included cleaning of mouth parts and antennae, rubbing of posterior legs with middle legs, lack of coordination of muscles as evidenced by difficulty in walking with gradual paralysis of posterior legs. While there were indications of general irritability there was little or no evidence of intense excitement as is characteristic of pyrethrum intoxication.

The grasshoppers were fed fresh tomato leaves daily. That the leaves were not rendered unfit for food for the grasshoppers is shown by the fact that in an experiment in which 50 nymphs were fed tomato leaves that had been treated for 24 hours with SO_2 107 p.p.m. the mortality was only 8 per cent in 11 days, while the mortality for an equal number of nymphs fed fresh tomato leaves for the same period averaged 12.5 per cent.

It is generally known that grasshoppers, when crowded or with an insuffcient food supply, may become cannibalistic. A careful check was made daily of the number in each cage for possible missing individuals or evidence of dismemberment of dead nymphs but these appeared not to be factors. The nymphs were distributed in groups of 5 and 25 depending upon the size of the cage. Ten nymphs in each series were caged individually. An average of 500 cc. of space was allowed per nymph. That cannibalism was not an important factor is shown by the fact that the mortality of 540 nymphs confined in groups in cages in the above experiments was 21.3 per cent, while the mortality of the 60 nymphs confined individually was 23.3 per cent. Since the mortality is higher for nymphs confined to individual cages than for nymphs caged in groups, cannibalism must have been a negligible factor.

Cockroaches

As grasshoppers were not available during the winter months, cockroaches were substituted in the SO_2 toxicity tests. Adults were collected in the bird house of the Bronx Zoological Garden. The species found proved to be a recent introduction of a tropical form which was identified by William T. Davis of Staten Island, New York, as *Leucophaea maderae* (Fabr.). Twenty adults confined in a wire cage were exposed to a concentration of 95 p.p.m. for 12 days in the SO_2 cabinet and a similar number in the control cabinet. An average of 875 cc. of space was allowed per adult. A kill of 80 per cent was obtained with SO_2 in 12 days. The mortality in the control was 35 per cent (Fig. 10). The high mortality rate may have been due in part to chilling of the cockroaches in transport.

The food supply consisted of oatmeal to which a small quantity of cornstarch (about 1 per cent by weight) had been added. Equal parts by weight of wheat bran and banana also were furnished. Water was supplied from a drop fountain. Two inverted saucers afforded shelter under which the roaches could hide.

Concentrations of 133 p.p.m. and 300 p.p.m. of SO_2 were tested on *Periplaneta americana* (L.) using the same experimental arrangement and technique as described for *Leucophaea maderae*. A mortality of 100 per

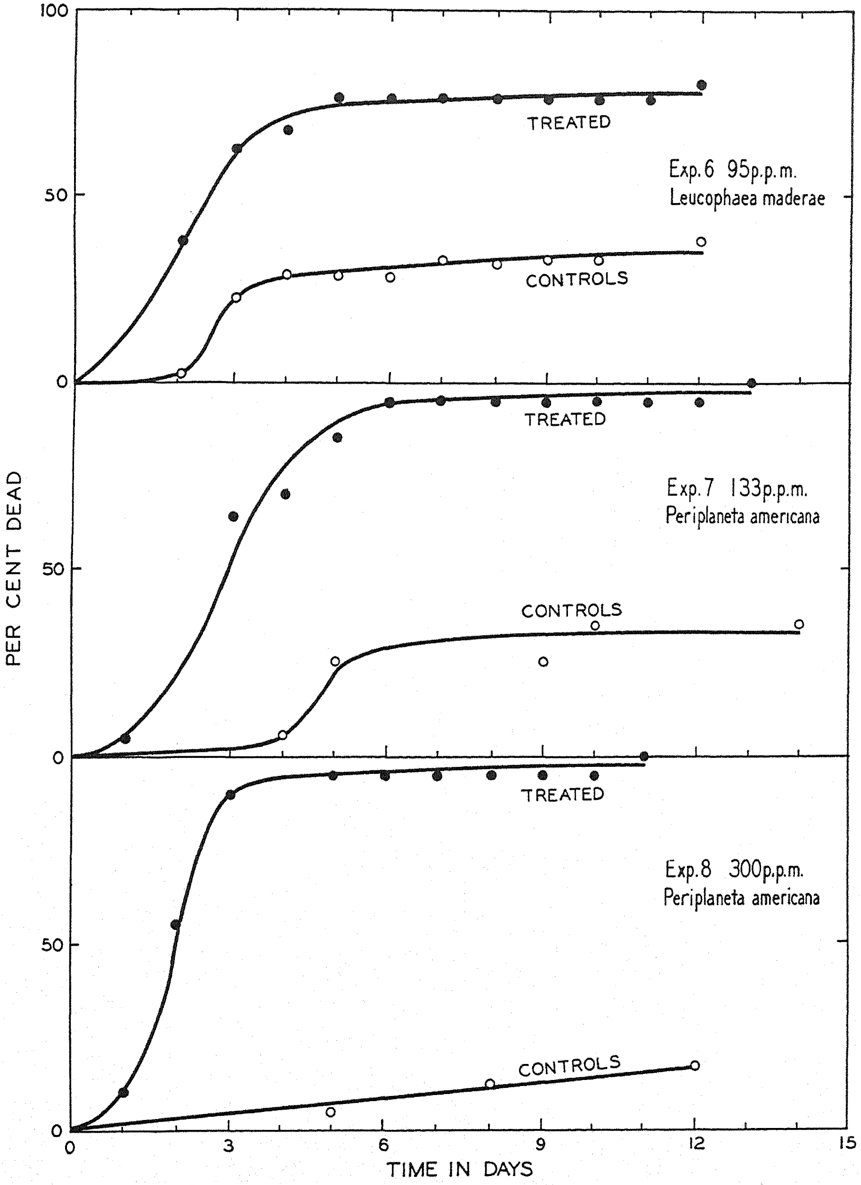

FIGURE 10. Mortality versus time toxicity curves for two species of cockroaches.

cent was reached in 12 days at a concentration of 133 p.p.m. At a concentration of 300 p.p.m. the kill was 55 per cent in two days, and 100 per cent in 11 days (Fig. 10). The mortality in the controls ranged from 16.6 per cent to 35 per cent.

It should be pointed out that the experimental conditions, so far as temperature and humidity were concerned, were arranged to meet the optimum conditions for guinea pigs and mice without regard for the optimum conditions for insects. Owing to the fact that repeated opening of the SO_2 cabinet would have seriously interfered with maintaining a uniform concentration of SO_2 in the cabinet, especially at the higher concentrations, counts of living and dead insects could not be made as frequently as desired.

DISCUSSION AND CONCLUSIONS

EVALUATION AND INTERPRETATION OF TOXICITY DATA

Because of the scarcity of experimental information regarding time-mortality curves, it is difficult quantitatively to evaluate the precision of the toxicity data which have been presented. If, however, the possibility of experimental error is excluded and only errors of random sampling are considered, an estimate of the reproducibility of the results can be obtained by the method of Bliss (2).

Using this method with simplifications similar to those suggested by Wilcoxon and McCallan (29) for dosage-mortality curves, it was found that time till death of 50 per cent of the guinea pigs in Exp. No. 9, for example, is reproducible within 19 per cent 19 times out of 20. Similar examination of the data for cockroaches at 300 p.p.m. shows reproducibility within 21 per cent.

Using Bliss's estimate of error it was further found that the difference in time till 50 per cent mortality of exercised and non-exercised guinea pigs in Exp. No. 9 was significant. The data of Table V suggest that there were no significant differences in susceptibility due to exercise at the lower concentrations.

The data of Table VII indicate no differences in susceptibility due to age or, incidentally, to weights of guinea pigs. The data summarized in Tables VI and XI show that pretreatments with sulphur dioxide did not significantly condition the susceptibility of guinea pigs or of mice, indicating that the effects of the gas are not necessarily residual or cumulative. This latter conclusion is supported by the data of Tables III and IV which show that a longer exposure was necessary to cause mortality in guinea pigs and in mice when the gas treatments were given in intermittent exposures with intervals free from gas.

Mice were found to be much more resistant than guinea pigs at concentrations of 150 p.p.m. and below, but were more susceptible at 300 p.p.m.

and much more susceptible at 1000 p.p.m., indicating a difference in slope of the dosage-time curves of the two species. The susceptibility of both grasshoppers and cockroaches (*Periplaneta americana*) approximated that of the mice. According to the findings of Zimmerman and Crocker (31) and others, plants are very much more susceptible to sulphur dioxide than were any of the animals studied. It is generally agreed that 1.0 p.p.m. will cause typical foliar markings within an hour on sensitive plants such as buckwheat (*Fagopyrum esculentum* Moench.) and alfalfa (*Medicago sativa* L.), growing under susceptible conditions.

In general the toxicity data reported in this paper are in agreement with the unpublished experiments of the Chemical Warfare Service (28) performed at the lower concentrations, but they are not in agreement with the army studies at concentrations above 100 p.p.m. Vedder and Armstrong report that the median lethal concentration for 24-hour exposure of guinea pigs was 404 p.p.m. The median lethal concentrations for mice were found to be 130 p.p.m. for 24 hours, 1350 p.p.m. for 10 minutes, 610 p.p.m. for 60 minutes, and 340 p.p.m. for a 360-minute exposure period.

The disagreement arises in part from the fact that the army data represent dosages which caused death after removal of the animals from the gas, while in the present paper the animals were allowed to remain in the gas until death. Further discrepancies are believed due to the inadequacy of the control and measurement of sulphur dioxide concentrations in the high-concentration experiments of the Chemical Warfare Service as well as to their methods of evaluating median lethal concentrations from widely scattered points on mortality-concentration curves.

EXTERNAL SIGNS AND SYMPTOMS

No signs of distress were shown by the animals while in the gas at 10 parts per million. At 25 and at 33 parts per million only a slight temporary dyspnea occurred which from other evidence may have been partly due to infection.

At a full-time concentration of 109 parts per million no unusual appearance could be made out in the guinea pigs for 12 hours; after this followed gradually lethargy, rhinitis, conjunctivitis, moderate dyspnea, weakness, distention of the abdomen, paralysis of the hind quarters, and death. The full-time mice were disturbed upon first introduction to the gas as evidenced by restlessness and rubbing of their noses, this state lasting for an hour, after which they appeared little affected for about 24 hours. After this only moderate lethargy, rhinitis, conjunctivitis, and dyspnea appeared, the mice being distinctly less affected than the guinea pigs. Distention of the abdomens of the living mice was difficult to observe. At 150 parts per million the signs showed by the full-time guinea pigs were the same except that the observation was made that dyspnea became more pronounced after feeding indicating, with the autopsy findings below, that pressure by the distended stomach upon the lungs and heart probably was an important factor in shortening the life of the animal. A short temporary period of distress was observed in these guinea pigs when they were first introduced into the gas. Again the mice, showing the same qualitative signs, were less affected by the gas than were the guinea pigs.

At 296 parts per million the signs were qualitatively the same in both guinea pigs and mice, but at 1014 parts per million the guinea pigs, both full time and alternate showed also moderate restlessness, lachrymation, coughing, sneezing, and frequent rubbing of noses. The mice were unusually active for a few minutes.

GROSS PATHOLOGICAL FINDINGS

The gross appearance post mortem of the organs of the animals exposed to the lower concentrations of gas did not differ greatly from those of control animals killed by ether or by a blow at the base of the brain; individual animals vary in the amount of congestion of all tissues, also small pleural hemorrhages are frequent in death from many causes; again small differences in the amount of distention of the lungs are of little value in diagnosis of pathologic change and the same is true of small differences of distention of the stomach due to varying amounts of food in the latter organ. In short, neglecting those experiments in which the animals obviously had contracted an infectious disease the only clearly unusual gross findings at the concentration of 33 parts per million or less were the distention of the gall bladder and slight to moderate pulmonary edema. At 10 parts per million even these findings were absent.

At 100 parts per million the cornea becomes opaque, the stomach becomes grossly distended, and congestion of the viscera more definite. At 150 parts per million a few animals show multiple gastric ulcers with hemorrhages, at 300 parts per million gastric distention, ulcers and hemorrhages are more pronounced, distention of the lungs less but massive hemorrhage of the lungs common. It may well be that these findings in the stomach and lungs are complementary as may be the acute dilatation of the right heart first found at this concentration. The minor phenomenon of distention of the gall bladder which has been constant is now less frequent especially in the mice but the time of exposure to the gas of course is less.

At 1000 parts per million the stomach is still grossly distended, but the ulcers are smaller, the hemorrhages less frequent, the gall bladder is less frequently distended, each suggesting a time factor in this short experiment. The lungs, which are greatly compressed by the stomach, are typically only moderately voluminous or red. Massive hemorrhage is less frequent in the lungs of the guinea pigs than at 150 parts per million, but more frequent in the mice. The heart is acutely dilated, particularly the right. The trachea is frequently hemorrhagic.

Marked (bullous) emphysema of the lungs was not observed at any concentration.

Animals receiving alternate exposure in each concentration of gas show fewer signs and fewer and less severe lesions, as would be expected, except at the highest concentration studied.

PROBABLE EFFECTS OF SULPHUR DIOXIDE ON HEALTH

The results of Exp. Nos. 2 and 5 clearly indicate that concentrations of sulphur dioxide such as prevail in city atmospheres have no direct effect on health. No mortality among healthy mice or guinea pigs resulted from 47 days of continuous treatment with 25.3 p.p.m. of SO_2; no abnormal symptoms of any sort developed during 30 days of continuous treatment of guinea pigs with 10.7 p.p.m. These results are substantiated by the data of Vedder and Armstrong (28) who found that guinea pigs, rabbits, and rats exposed to 10 p.p.m. showed normal physiological development during 90 days of continuous exposure. The data of the Air Hygiene Foundation (14) suggest that the city-dweller living continuously in the center of a highly industrialized city such as St. Louis is subjected to concentrations higher than 2 p.p.m. less than 20 days during a lifetime of 60 years.

The possibility that sulphur dioxide has an indirect bearing on health by effecting a predisposition to a disease or infection cannot be evaluated from the data given in this paper. While mortality of mice from epizootic enteritis was greater in the treated mice in Exp. No. 3, mortality was greater in the controls in Exp. No. 5. One group of Exp. No. 2 mice showed a greater mortality among the treated, the other group showed a greater mortality among the control mice. Seven guinea pigs suffering from epizootic pneumonia died in Exp. No. 4, but there were four deaths among the controls. Vedder and Armstrong (28) report that the mortality among SO_2-exposed rats accidentally infected with B pseudo tuberculosis was less than among control rats similarly infected.

The fact that the guinea pigs and mice were able to withstand concentrations as high as 150 p.p.m. for considerable periods of time when exposed for only eight hours per day suggests that no direct toxic effects result to industrial workers exposed occasionally to concentrations of this magnitude. This is in agreement with the clinical findings of Kehoe, Machle, Kitzmiller, and LeBlanc (11).

The results of Exp. Nos. 3, 5, and 6 cast serious doubt on the findings of the commission investigating the Meuse Valley disaster (4) which ascribed the toxicity of the prevailing fog to sulphur dioxide and its oxidation products. The commission estimated that the maximum concentration which could have prevailed at any time during the fog was 38 p.p.m., if no allowance is made for adsorption of the gas by vegetation, soil, fog droplets, etc. It is likely that at least 75 per cent of the gas was so adsorbed, but even the theoretical maximum of 38 p.p.m. could probably have been endured for several days without toxic effect. It must be noted, however, that the possibility of some harmful effect from this concentration of SO_2 cannot be ruled out without experimental studies with a foggy atmosphere such as prevailed in the Meuse Valley at the time of the disaster.

While the results reported in this paper show that sulphur dioxide is considerably less toxic than most observers have believed, they cannot be construed to support such extravagant claims as those of Rawlins (20) who recommends the use of sulphur dioxide in the treatment of the epidemic cold. Rawlins argues that since 6000 p.p.m. of SO_2 kill the virus causing hoof and mouth disease, and since the epidemic cold may be caused by a virus, therefore SO_2 should cure the epidemic cold. He neglects the possibility that SO_2 may kill the patient before it will kill the virus.

SUMMARY

A series of experiments has been conducted to test the effects on vertebrate and invertebrate animals of prolonged exposure to controlled concentrations of sulphur dioxide. Guinea pigs, mice, grasshoppers, and cockroaches were used as experimental material. Concentrations of 10, 25, 35, 65, 100, 150, 300, and 1000 p.p.m. (in round numbers) were tested.

In general, resistance to the gas was greater than had been expected, and signs of distress were less. No significant mortality or signs of distress occurred among healthy animals at concentrations of 33 p.p.m. or below. Neither age of the animals nor pretreatment with sulphur dioxide effected susceptibility to the gas. Exercise disposed a greater susceptibility to 1000 p.p.m. but had no significant effect at lower concentrations. A longer exposure to the gas was necessary to cause mortality when the SO_2 treatments were given in intermittent doses.

At concentrations of 150 p.p.m. and below, mice were more resistant than guinea pigs, but they were more susceptible at concentrations of 300 p.p.m. and 1000 p.p.m. indicating a difference in slope of the dosage-time curves of the two species. The susceptibility of both grasshoppers (*Melanoplus differentialis*) and cockroaches (*Periplaneta americana*) approximated that of the mice.

Signs in living exposed vertebrate animals at the higher concentrations included lethargy, rhinitis, lachrymation, coughing, conjunctivitis, moderate dyspnea, distention of the abdomen, weakness, and paralysis of the hind quarters.

Signs in invertebrate animals at the highest concentration included cleaning of mouth parts and antennae, lack of coordination of muscular movements, and paralysis of posterior legs.

Pathologic changes in vertebrates include general visceral congestion of slight to moderate degree, slight to moderate edema of the lungs with hemorrhages at higher concentrations, acute dilation of the right heart at higher concentrations, gross distention of the stomach with multiple ulcers and hemorrhages at the higher concentrations, distention of the gall bladder except at lowest concentrations.

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INHIBITION OF THE GROWTH OF BUDS OF POTATO TUBERS WITH THE VAPOR OF THE METHYL ESTER OF NAPHTHALENEACETIC ACID[1]

JOHN D. GUTHRIE

The marked inhibiting action of naphthaleneacetic acid on the growth of the buds of potato tubers has been reported in previous papers (7, 8, 9). Most of this work was with cut pieces of the tubers, although inhibition was obtained with whole tubers by inserting toothpicks soaked in the chemical into the tubers. A spray consisting of 1 g. naphthaleneacetic acid, 50 cc. olive oil, and 1 liter of acetone was effective when the tubers were stored at 10° C. Since it seemed likely that the best way to apply an inhibiting substance to the whole tubers would be as a vapor, the methyl ester of naphthaleneacetic acid was tried, because it was thought that it might be volatile enough to inhibit the buds. This proved to be the case.

Potato tubers (*Solanum tuberosum* L.) of the Irish Cobbler variety that had been harvested in New Jersey early in November 1938 and stored in a cool cellar were placed in two 6-liter desiccators. The treatment was started on February 11th by slowly drawing air through saturated sodium carbonate solution to control the humidity and then through a tube containing a roll of filter paper moistened with 420 mg. of the methyl ester of naphthaleneacetic acid. This air was drawn continuously through the desiccator containing the tubers. The temperature ranged from 25° to 28° C. After 27 days 6 buds had started to grow on the 12 treated tubers, while 59 buds had started to grow on the control tubers that had been treated similarly with air passed over plain filter paper. Typical tubers of this experiment are shown in Figure 1. At this time the treated tubers were divided into two lots and one lot treated with ethylene chlorohydrin by the method of Denny and Miller (4). After 10 more days 21 sprouts had started on the 6 tubers treated with ethylene chlorohydrin and 5 sprouts on the 6 tubers that were not treated with ethylene chlorohydrin. This is in agreement with previous results obtained with cut pieces (8).

The effect of wrapping tubers in paper impregnated with the methyl ester was tried. A solution of 180 mg. of the ester in 10 cc. of acetone was poured on six 11-cm. filter papers and allowed to evaporate. Small potato tubers were then wrapped in these papers and placed in a beaker covered with a watch glass. There were no buds growing on these tubers 23 days later, while 34 buds were growing on 6 control tubers wrapped in papers moistened with acetone. In another experiment a solution of 200 mg. of the ester in 7 cc. of acetone was poured on four 11-cm. filter papers. After

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[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 189.

the acetone had evaporated, the papers were placed on top of 6 tubers in a 1-liter beaker, covered with a watch glass and sealed with clay. The clay was removed after 24 hours, but the papers were left in the covered beaker. After 11 days there were 18 sprouting buds on the 6 treated tubers, while 51 buds were growing on 6 control tubers treated similarly with filter paper moistened only with acetone.

The effect of storing tubers in ordinary paper bags impregnated with the ester was investigated. A 5-lb. brown paper bag was sprayed inside with acetone containing 480 mg. of the ester. After the acetone had evaporated, 6 tubers were placed in the bag, the top folded over and fastened with paper clips. The bag was then placed in a 12-lb. bag which was folded over and fastened. After 11 days only 3 buds were growing on the 6 treated tubers while 24 buds were growing on 6 control tubers stored in a similar way in a bag sprayed with acetone.

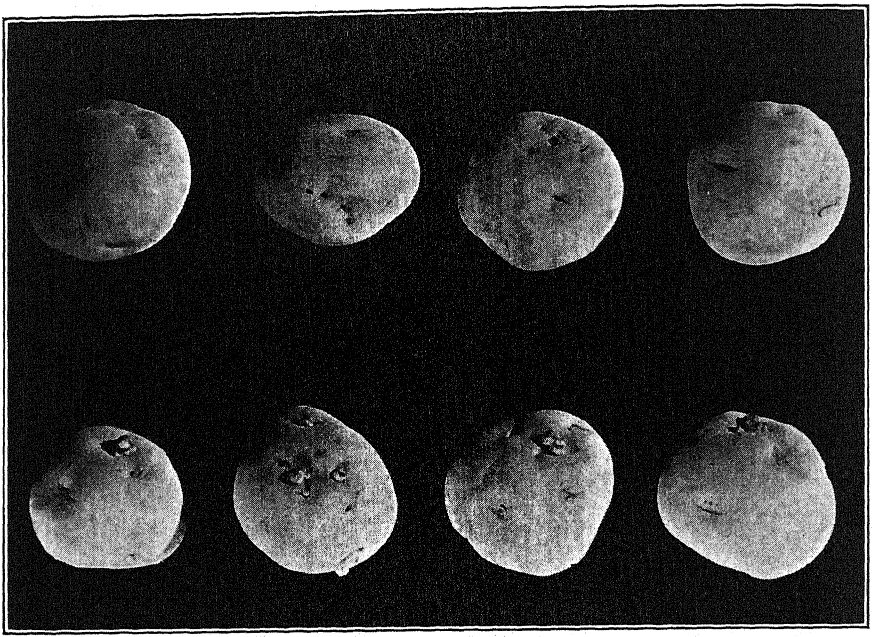

FIGURE 1. Upper row, tubers treated with the vapor of the methyl ester of naphthaleneacetic acid. Lower row, control tubers.

In order to show in another way that the methyl ester of naphthaleneacetic acid was volatile, one drop of an acetone solution of the ester, 680 mg. per 25 cc., was placed on filter paper. After the acetone had evaporated, this was placed under a bell jar with a tomato plant. Epinasty began after about 2 hours and was marked after 24 hours. A control plant showed no epinasty. This experiment was repeated. The filter paper that had been used in the experiment described at the beginning of this report produced marked epinasty when placed under a bell jar with a tomato plant. It also inhibited sprouting when placed in a covered beaker with a second lot of potato tubers. Zimmerman, Hitchcock, and Wilcoxon (17), and Zimmerman and Hitchcock (15) have shown the methyl ester of naphthaleneacetic acid to cause epinasty of tomato leaves when applied in lanolin and in water to the stems and petioles.

These experiments definitely place the methyl ester of naphthaleneacetic acid in the class of substances causing epinasty in the vapor form. Other substances known to do this are: ethylene, acetylene, propylene, butylene, and carbon monoxide (2); ethyl bromide, ethyl iodide, and propyl chloride (3); acetonitrile (14); and *cis* cinnamic acid and phenylacetic acid (16). The bud-inhibiting action of auxin-like substances has been shown by Thimann and Skoog (13) and by Hitchcock (10). Ethylene also inhibits the growth of buds of potato tubers as shown by Elmer (5), Gane (6), and Huelin (11). Miller (12) has noted that the sprouting of potato tubers is inhibited by solutions of thioacetamide.

The methyl ester of naphthaleneacetic acid may prove useful in retarding the sprouting of the buds of potato tubers and of other plants. Although the amount taken up by potato tubers when exposed to the vapor of the methyl ester may be very small, this must be determined before its use on potato tubers intended for food can be considered safe, since intraperitoneal toxicity of naphthaleneacetic acid has been shown by Anderson, Shimkin, and Leake (1).

SUMMARY

The methyl ester of naphthaleneacetic acid inhibits the growth of buds of potato tubers, and is sufficiently volatile at room temperature (25° to 28° C.) that it can be introduced into intact tubers in the vapor form. The sprouting of whole tubers can be retarded by merely storing them in the presence of paper impregnated with the ester. The growth of the buds was successfully inhibited by storing the tubers in a paper bag, the inside of which had been sprayed with the chemical.

The methyl ester of naphthaleneacetic acid also induces epinasty of tomato leaves when a piece of filter paper containing a small amount of the substance is placed in a bell jar with the plant.

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THEORETICAL PRINCIPLES UNDERLYING LABORATORY TOXICITY TESTS OF FUNGICIDES[1]

FRANK WILCOXON AND S. E. A. MCCALLAN

The principles involved in laboratory toxicity experiments on fungicides are closely related to those involved in the determination of the potency of drugs by the methods of bio-assay. Burn (4, p. 146) in 1930 stated that "Biological assay, as carried out by the majority of workers in the world, still remains a subject for amusement or despair, rather than for satisfaction and self-respect." In the same paper he speaks of "certain principles which during the past few years have been shown to be capable of transforming this whole subject from the plane of an insidious means of self-deception to that of a well-ordered and progressive science." The work of Trevan (8), Gaddum (6), and recently that of Bliss (1, 2, 3) have contributed materially to the accomplishment of such a transformation.

NATURE OF THE PROBLEM

Toxicity experiments may be divided into two general types. In experiments of the first type the response of the organisms to the toxic agent is measured quantitatively, as for example where the diameter of fungal colonies is measured, or the weight of mycelial mats. In experiments of the second type the organisms being tested are divided into two groups depending upon whether a given individual has or has not exhibited a definite observable response to the toxic agent. Such a division might be into germinated or non-germinated spores in the case of the usual spore germination tests. This type of experiment is said to be one involving a "quantal response," and is the type with which this paper is concerned.

When different samples from the same lot of spores are subjected to various concentrations of the toxic agent the proportion of the spores showing a response, that is failing to germinate, increases with increasing concentration of the toxic agent. We do not find that all the spores germinate in toxic solutions up to some particular concentration, and that they all fail to germinate in a slightly higher concentration. As a result of this behavior one must conclude that individual spores have different lethal concentrations, and that there is a distribution of individual lethal doses just as there is a distribution of weights or lengths of the spores. It is not usually possible to determine directly the distribution of individual lethal doses in toxicity experiments, but the important features of the distribution may be inferred from the toxicity data obtained.

As a result of the examination of a large number of toxicity experiments

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[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 190.

with many different poisons and with many species of plants and animals, it has been concluded that the distribution of individual lethal doses tends to be symmetrical when plotted against the logarithm of the dose (6, p. 11). Such a distribution is shown in Figure 1. The important features of such a distribution are the mean or median, *m*, which are the same if the curve is symmetrical and the standard deviation, λ, which is a measure of the spread of the distribution. The wider this spread the more unlike are the individual spores in regard to their lethal doses.

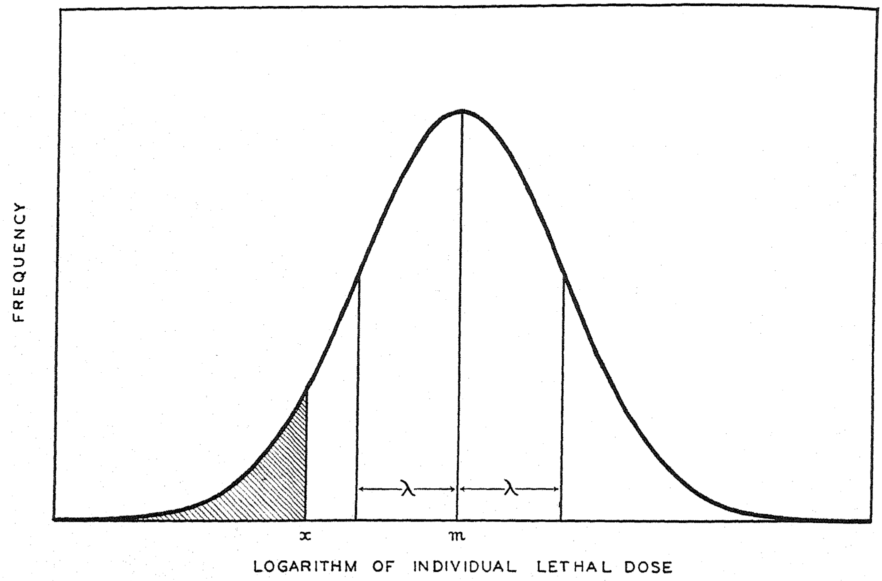

FIGURE 1. Hypothetical distribution curve of individual lethal doses. Mean of individual lethal dose is *m* and standard deviation is λ. All spores having an individual lethal dose less than concentration *x* fail to germinate.

The height of the curve above the base at any particular concentration value represents the relative number of spores having that particular concentration for their individual lethal dose. The relation of this curve to the usual data of a toxicity experiment may be understood if we consider the result of applying some particular concentration of toxic agent such as *x* to this population. It is obvious that all spores having an individual lethal dose of *x* units or less will fail to germinate. The percentage failing to germinate will be represented by the shaded area under the curve to the left of *x*.

The problem in toxicity determinations is to estimate the value of the dose killing or inhibiting 50 per cent of the spores, that is, the median lethal dose or LD50 (8), as well as the limits within which it may be expected to lie with any desired degree of probability such as odds of 20:1. This estimate must be derived from a series of observations of percentage germination at several concentrations of toxic agent, some of which may be quite far removed from the LD50 value.

METHODS

Use of semi-logarithmic paper. Various methods are available for estimating the LD50 and its limits of error depending upon the degree of accuracy desired. One of the simplest is to plot the percentage of viable spores which fail to germinate against the logarithm of the concentration of the toxic agent. In order to avoid the use of logarithms, semi-logarithmic paper may be used. The points will usually lie more or less on a sigmoid curve and such a curve may be drawn free-hand among the points and the concentration giving 50 per cent germination may be estimated.

The results shown below were obtained using spores of *Macrosporium sarcinaeforme* (Cav.) and a copper fungicide at several different concentrations. The ratio between successive concentrations was 1.5 in this case, and 100 viable spores were counted at each concentration.

Concentration of copper in per cent	0.198	0.296	0.444	0.667	1.000	1.500	2.250	3.375
Per cent spores non-germinated	0	1	6	11	56	82	94	100

These results have been plotted on semi-logarithmic paper in Figure 2 A and the LD50 estimated as 0.98 per cent copper. The value of λ, which is a measure of the extent to which the spores vary in their individual lethal doses, was estimated by drawing horizontal lines to the curve from 16 and 84 per cent and projecting down to the concentration axis. The concentration value corresponding to 84 per cent was 1.58 per cent copper, and this divided by the LD50 gave a ratio of 1.61. The value corresponding to 16 per cent was 0.64, which divided into the LD50 gave 1.53. Averaging these two values we get 1.57 as the estimate of λ. It will be observed that in this case the value of λ is not very different from that of the dose ratio, d, i.e., the ratio of successive concentrations, which is 1.50. In the case of a homogeneous population it is possible to form an approximate estimate of the range within which the LD50 may be expected to lie 19 times out of 20 in the following manner. The values of λ and d may be averaged giving 1.535. This number is then raised to a power whose exponent is $1.46/\sqrt{n}$, where n is the number of spores used at each concentration, that is, 100 in this case; 1.535 must then be raised to the 0.146th power. This may be done on a Log Log slide rule or by multiplying the logarithm of 1.535 by 0.146. The result is 1.065 which indicates that 19 times out of 20 the LD50 would be expected to fall within $0.98/1.065$ and 0.98×1.065. These limits correspond to 0.920 and 1.044 per cent

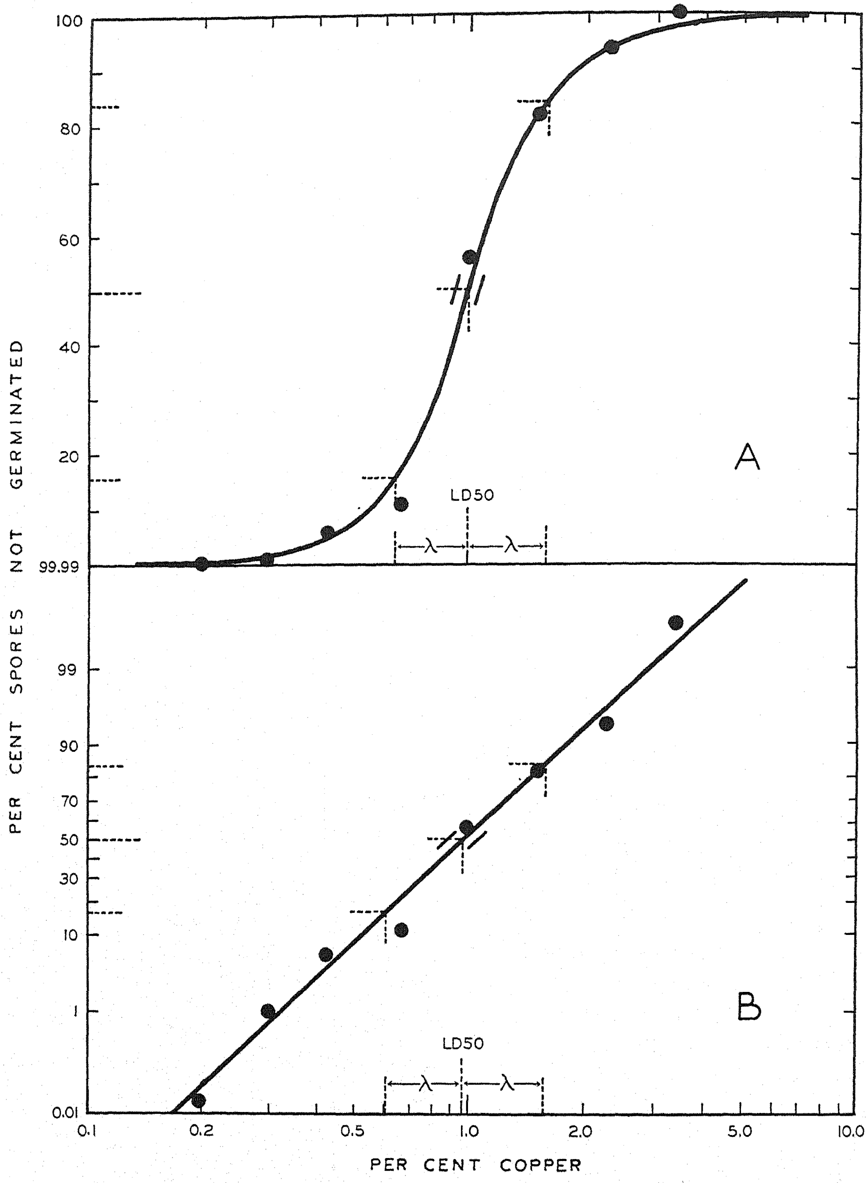

FIGURE 2. Toxicity curves for action of a copper fungicide on spores of *Macrosporium sarcinaeforme*. A. Per cent non-germination plotted against logarithm of concentration. B. Same data plotted on logarithmic probability paper. Heavy parallel lines indicate zone within which the LD50 may be expected to fall 19 times out of 20, under the same conditions.

copper. Table I is designed to facilitate this computation. It gives the values of numbers from 1.1 to 5.9 raised to a power whose exponent is 0.146.

TABLE I

FACTORS FOR OBTAINING UNDER SPECIFIED CONDITIONS,* THE 19/20 LIMITS OF ERROR OF THE LD50 AND LD95. FACTORS IN BODY OF TABLE AND AVERAGE OF λ AND DOSE RATIO IN MARGINS

LD50

	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
1	—	1.01	1.03	1.04	1.05	1.06	1.07	1.08	1.09	1.10
2	1.11	1.12	1.12	1.13	1.14	1.14	1.15	1.16	1.16	1.17
3	1.17	1.18	1.19	1.19	1.20	1.20	1.21	1.21	1.22	1.22
4	1.23	1.23	1.23	1.24	1.24	1.25	1.25	1.26	1.26	1.26
5	1.26	1.27	1.27	1.28	1.28	1.28	1.29	1.29	1.29	1.30
					LD95					
1	—	1.02	1.05	1.07	1.09	1.11	1.13	1.15	1.17	1.18
2	1.20	1.21	1.23	1.24	1.26	1.27	1.28	1.29	1.31	1.32
3	1.33	1.34	1.35	1.36	1.37	1.38	1.39	1.41	1.42	1.43
4	1.43	1.44	1.45	1.46	1.47	1.48	1.49	1.50	1.50	1.51
5	1.52	1.53	1.54	1.54	1.55	1.56	1.56	1.57	1.58	1.59

* Homogeneous population; 100 spores counted at each concentration; dose ratio substantially equal to λ.

Although the method described above may serve for a rough approximation to the best estimate of the LD50, there are several sources of error involved. Probably no two investigators would agree on the best curve to be drawn among the points, and it is also a fact that the various points do not have equal weight in locating the LD50. It is a difficult matter to draw a curve free-hand and give each point its proper weight.

Use of logarithmic probability paper. A type of graph paper has been designed,[2] however, which permits the plotting of the usual sigmoid toxicity curve as a straight line. This paper is called "logarithmic probability paper" and may be used in the following manner: The various percentages of spores failing to germinate are plotted on the vertical axis against the corresponding concentrations on the horizontal axis, and the best straight line is drawn through the points giving the greatest weight to points in the neighborhood of 50 per cent. The LD50 concentration is then estimated as in the previous method.

The previous data, plotted in this manner, are shown in Figure 2 B. It will be noticed that the vertical scale on this type of graph paper does not extend to germination values of 0 or 100 per cent. While these percentages usually contribute comparatively little information toward the estimation of the LD50 it is desirable to make use of them in many cases. Owing to the fact that a limited number of spores are counted at each

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[2] Codex Book Co. Inc., Norwood, Massachusetts.

point the concentration corresponding to 0 per cent germination will usually be underestimated while that corresponding to 100 per cent germination will usually be overestimated. Methods have been worked out for correcting these values and combining the information which they furnish with that furnished by the points between 0 and 100 (1, 3). If a transparent triangle or straight edge be placed along these latter points in such a way as to indicate what appears to be the best straight line, then an expected value can be read for the concentrations which actually gave 0 and 100 per cent, at the point where the straight edge crosses these concentrations. The corrected values depend upon these expected values, as shown in Table II. In the example above, at a concentration of 3.375, 100 per cent non-germinated spores were observed. The expected value as read from the line in Figure 2 B is 99.57. Reference to Table I shows that the corrected percentage corresponding to this value is 99.85, and this latter value is actually plotted in Figure 2 B. With the use of this logarithmic probability paper the LD50 was estimated as 0.96 per cent copper instead of 0.98 as previously, while the value of λ was 1.60 compared with the previous value of 1.57.

TABLE II

CONVERSION OF EXPECTED PERCENTAGES TO CORRECTED PERCENTAGES (BODY OF TABLE) FOR USE WITH LOGARITHMIC PROBABILITY PAPER

	0	1	2	3	4	5	6	7	8	9
50	89.5	89.5	89.6	89.6	89.6	89.7	89.7	89.8	89.9	90.0
60	90.1	90.2	90.4	90.5	90.7	90.8	91.0	91.2	91.4	91.6
70	91.7	91.9	92.2	92.4	92.6	92.8	93.0	93.3	93.5	93.8
80	94.0	94.3	94.5	94.8	95.1	95.3	95.6	95.9	96.2	96.5
90	96.8	97.1	97.4	97.7	98.0	98.4	98.7	99.0	99.3	99.7
	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
99.5	99.83	99.83	99.83	99.84	99.84	99.84	99.85	99.85	99.85	99.86
99.6	99.86	99.86	99.87	99.87	99.87	99.88	99.88	99.88	99.89	99.89
99.7	99.89	99.90	99.90	99.91	99.91	99.91	99.92	99.92	99.92	99.93
99.8	99.93	99.93	99.94	99.94	99.94	99.95	99.95	99.95	99.96	99.96
99.9	99.96	99.97	99.97	99.97	99.98	99.98	99.98	99.99	99.99	99.99

Estimation of LD95. In some cases it may be desirable to estimate the LD95 or the concentration which would be expected to prevent 95 per cent of the spores from germinating. This value may be read from the line which has been fitted to the observed points on logarithmic probability paper in the same manner as the LD50 giving 2.17 per cent copper. The zone of error within which it may be expected to lie 19 times out of 20 will in this case be considerably wider than in the case of the LD50. For a homogeneous population its value may be estimated in a manner similar to that used for the LD50. The average of λ and d is raised to a power whose exponent is $2.60/\sqrt{n}$, in this case 0.26; thus, $1.535^{0.26}$ is 1.118. That is, the LD95 value should fall within 2.17/1.118 and 2.17×1.118. These limits are 1.94 and 2.43 per cent copper. The factors for obtaining the 19/20 zone of error for the LD95 are given in Table I.

Significance of difference of two toxicity curves. If it is desired to estimate whether the LD50 or LD95 values of two compounds differ significantly, the following approximate method may be used. The data are plotted on logarithmic probability paper, straight lines fitted, and the 19/20 zones of error marked on the graph as described above. If these zones do not overlap, the compounds are clearly different. If the distance of the overlap as measured by dividers or a ruler does not exceed 0.3 of the average width of the zones similarly measured, the compounds differ with odds of 20:1 or more. The compounds must have been tested at the same time and under the same conditions.

Correction for extraneous sources of error. The methods illustrated for obtaining the width of the 19/20 zone apply to experiments in which the population is homogeneous and in which conditions can be so controlled that extraneous sources of error are negligible. If this is not the case the width of the 19/20 zone will be greater than that calculated by these methods. In spore germination experiments an important source of error arises from the fact that the concentration of fungicide to which the spores are exposed may not be exactly known. It is not possible to spray slides twice alike, and if the time of spraying is taken as a measure of the applied amount, then the actual amount on the slide may differ from that which is assumed to be present. If the time of spraying is fixed and various dilutions of the spray material are used, then an appreciable error arises from the difficulty of diluting and sampling spray materials accurately (7).

It is possible to test whether extraneous sources of variation are present by means of the chi-square test (χ^2). If the results have been plotted on logarithmic probability paper and a straight line has been fitted, χ^2 may be estimated as follows, if 100 spores are counted at each point. At each point on the graph the difference between the found percentage of non-germinating spores and the expected percentage as read from the plotted line is calculated. This difference is squared and divided by the product of the expected percentage of germinated and of non-germinated spores, the result multiplied by 100 gives χ^2 for that particular point. Figure 3 has been prepared to facilitate the computation of χ^2. The sum of the χ^2 values obtained at the various points gives χ^2 for the line as a whole. The degrees of freedom (n) will be 2 less than the number of points used in plotting the line. If a particular laboratory using a given technique has obtained a record of a number of such χ^2 values, with their associated values of n, then these values may be added together and the values of n likewise added. The value of $\sqrt{S(\chi^2)/S(n)}$ gives a factor by which the width of the 19/20 zones should be multiplied in order to allow for ex-

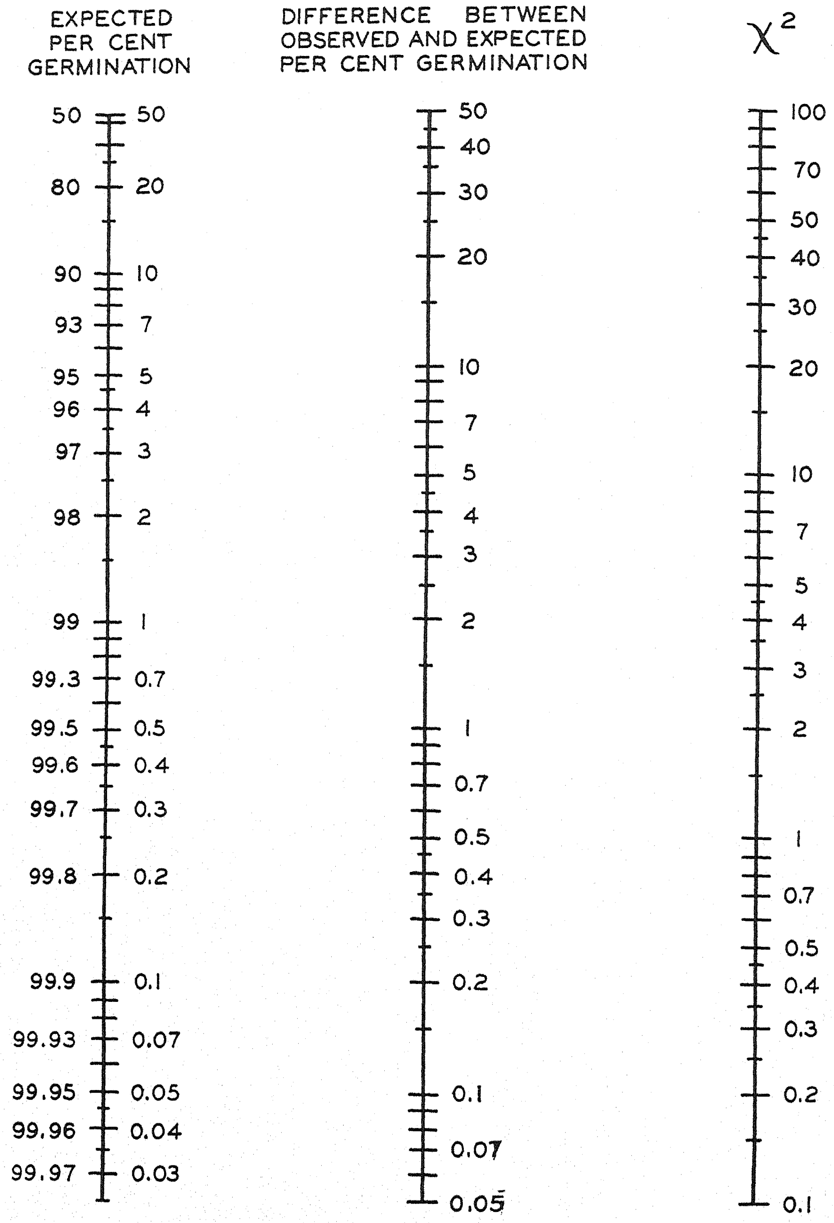

FIGURE 3. Nomograph for obtaining χ^2 values for points of a toxicity curve, 100 spores being counted at each point. A straight edge placed across the three vertical scales will connect the expected per cent, the difference between observed and expected, and the corresponding value of χ^2. For example, if 80 per cent is the expected germination (or non-germination) as read from the toxicity curve, while the observed value is 84 or a difference of 4 per cent, χ^2 will be 1.0.

traneous sources of error. In multiplying the 19/20 zone by this factor, its actual width as plotted on the logarithmic probability paper is measured with a millimeter scale and a pair of dividers, no attention being paid to the units as printed on the bottom of the paper. This width in millimeters is multiplied by the factor and the limits of the corrected zone marked on the paper. Data accumulated in this laboratory and elsewhere on spore germination tests with spray materials indicate that this factor may have a value of about 2.0.

More precise methods. The most precise method of fitting the best straight line to the points observed is given by Bliss (1). It involves the transformation of the observed percentages to units called probits, while the concentrations are changed to logarithms. The line is fitted mathematically by the method of least squares, giving the proper weight to the points. The value of the LD50 is calculated as well as the width of the zone within which it should lie with a probability of 19/20, and thus errors in drawing the line and estimating from graph are eliminated. The necessary tables for doing this may be found in Fisher and Yates (5). A calculation of the LD50 from the data above by this more precise method gave a value of 1.0063 per cent copper which is somewhat higher than that given by the other two methods. The 19/20 zone is 0.945 and 1.072 per cent copper while the approximate method described above gives 0.92 and 1.044.

USE OF A STANDARD FUNGICIDE

One of the difficulties encountered in the laboratory testing of fungicides arises from the fact that values of the LD50 obtained in experiments run at different times or in different laboratories may vary widely. One obvious reason for this is the fact that spores from a different transfer must be used at different times. These variations are usually greater than would be expected from the zone of error estimated from the results of a single experiment (7).

A considerable improvement can be effected, however, if the material undergoing test is rated in terms of a standard fungicide, such for example as Bordeaux mixture. The LD50 for the unknown is divided into that obtained for the standard in the same experiment, and the quotient is a ratio which is relatively independent of the circumstances surrounding a particular experiment. According to Burn (4, p.146), "While a biological reaction may be used in order to compare the strength of two preparations one with the other, it cannot be used by itself to define the potency of one preparation alone." As an example of the use of a standard, the following values were obtained for the LD50 of a certain fungicide on the spores of *Sclerotinia fructicola* (Wint.) Rhem. in five experiments at different times: 57, 88, 185, 114, 150, expressed as p.p.m. of copper. The standard gave the values 23, 62, 98, 50, 58. By dividing each value of the standard by the corresponding value of the unknown, we obtain the coefficients 0.403, 0.705, 0.530, 0.438, and 0.387. These coefficients agree much better than the LD50 values from which they were derived, showing that the use of a standard has tended to reduce part of the variation encountered in different experiments.

SUMMARY

Toxicity experiments may be classified into two types: (a) those in which some property of each individual is measured quantitatively such as germ tube length, diameter of colonies, etc.; (b) those in which the individuals are divided into two categories such as germinated and non-germinated spores.

The toxicity curves obtained in method (b) are the result of the fact that each individual spore has its own particular lethal dose, and there is a distribution of individual lethal doses which is usually normal when plotted against the logarithm of the concentration.

The problem in toxicity experiments is to deduce the properties of the curve of individual lethal doses from the toxicity data obtained. Two rapid approximate methods of doing this are described and examples given. These methods lead to an estimation of the LD50 value, that is, the concentration preventing 50 per cent germination, and the range within which it may be expected to lie 19 times out of 20. The methods may be extended to provide an estimate of the LD95 and its corresponding zone of error if desired.

When fungicides are to be compared which have been run at different times or in different laboratories, they should be rated in terms of a standard which is run at the same time as the unknown. In this way errors due to many obscure causes may be reduced.

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OXYGEN REGULATES THE DORMANCY OF THE POTATO[1]

Norwood C. Thornton

The generally accepted explanation of the inability of freshly-harvested potato tubers to sprout when replanted at once after harvest is that the buds do not get enough oxygen from the air, that the peel is at first impermeable to this gas, and that germination of buds becomes possible only later when the peel becomes more permeable and permits the entrance into the tuber of a sufficient supply of oxygen. The corollary to this view is that the normal oxygen content of the air (20 per cent) would be sufficient and possibly the optimum for germination if the buds could get it.

The present paper shows that the failure of the potato buds to grow is due to the oxygen content of the air being too high, not too low, and that growth can be initiated at once if the oxygen concentration is reduced from 20 to approximately 2 to 10 per cent, and further, that the peel of the freshly-harvested tuber is not impermeable to oxygen but is, in fact, more permeable at that time than at any later stage, that its permeability decreases, not increases, with time, until a point is reached at which the supply of oxygen to the buds has been sufficiently reduced to furnish these low concentrations which are required for sprouting to proceed. The young tuber has no membrane that will inhibit the passage of oxygen and it is not until the later stages of its life span that the skin becomes dense, more cells are present, and the cell walls become thicker. The natural growth of this membrane to a point at which entrance of oxygen is restricted marks the end of the period of rest of the tuber and furnishes the conditions for the development of new plants. If wounded (by peeling the surface or cutting into pieces) dormant tubers will sprout, not because of a mechanical facilitation of the entry of oxygen as had been previously supposed, but because of a retardation in the entry of oxygen by the formation of dense "wound cork" tissue that the tuber rapidly develops.

MATERIAL AND METHODS

Potato (*Solanum tuberosum* L.) tubers of the varieties Irish Cobbler, from lots harvested either in North Carolina, New Jersey, or the Institute gardens, Bliss Triumph and Golden from the Institute gardens, were used. The potatoes were subjected to treatments immediately upon being harvested and after being held at 10°, 20°, or 23° to 28° C. for 10 to 25 days after harvesting. Both whole tubers and tubers cut into one-eye pieces were used; and whole or cut tubers were also held with skins intact and

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[1] Awarded an A. Cressy Morrison Prize, in 1938, by the New York Academy of Sciences.

with skins previously removed by peeling or scraping. Both moist and relatively dry conditions were maintained during the period of treatment. The moist condition was obtained by covering the tubers with moist sphagnum moss and the relatively dry condition was obtained by holding the tubers in a closed container without the moss, so that the tubers remained dry yet exposed to a high relative humidity. Also, potatoes were held in an open room at 23° to 28° C. where they were exposed to approximately 50 per cent relative humidity.

The potatoes were treated at atmospheric pressure with pure oxygen and nitrogen and with gas mixtures consisting of various percentages by volume of oxygen and nitrogen. The procedure of treatment of the potatoes with the gas mixtures containing from 0 to 100 per cent of oxygen was the same as that previously described (11); the gas mixtures were made up and passed into and through 8-liter tin containers. The gas mixtures were renewed each day in order to prevent an exposure of the tubers to a supply of oxygen lower than desired. Gas analyses of the storage atmosphere were made at frequent intervals to insure that the oxygen supply was being held at the desired concentration. The carbon dioxide produced through respiration of the tubers was absorbed by a 4 per cent solution of sodium hydroxide. This concentration of alkali had little effect upon the relative humidity of the storage atmosphere. This precaution was considered necessary in the light of previous work (12) in which it was found that carbon dioxide was effective in breaking the dormancy of freshly-harvested potatoes.

To obviate any considerable change in the oxygen and carbon dioxide content of the container during a 24-hour period it was necessary to have the volume of the container large in proportion to the number of tubers. For this reason the number of tubers in each test had to be limited to 4 or 6.

The treatments were carried out at room temperature, which varied from a minimum of 23° C. to a maximum of 28° C. during the spring, summer, and autumn months.

Observations were made not only on the production of visible sprouts at the eyes of the intact tuber during treatment, but also on the ability of the treated tubers or pieces of tubers to produce sprouts in soil in a basement room at 23° to 28° C., also on the growth of plants in soil in the greenhouse, and in the open field at the usual summer temperatures.

Although the nitrogen treatment (absence of oxygen) was not the most effective or desirable from the standpoint of breaking the dormancy of the potato, it was necessary to employ this procedure as a control for the oxygen treatments. The nitrogen was scrubbed free of oxygen with alkaline pyrogallol and washed with water before being introduced into the container with the potatoes. This treatment is rather drastic and must be carried out with caution in order to prevent breakdown of the tissue.

Tubers held in a moist condition in nitrogen developed injury more readily than tubers held in a relatively dry condition. Only tubers with intact skins could be used and the nitrogen had to be replaced every 24 hours. If the nitrogen was not replaced within 3 days with the moist tubers or within 6 or 8 days with the relatively dry tubers a breakdown of the tissue occurred, due, no doubt, to the accumulation of products of respiration and a more rapid growth of organisms as a result of this condition. Some tests with potatoes in nitrogen have been conducted for as much as 4 weeks without development of rot. Freshly-harvested immature potatoes must be allowed to dry a few days before being subjected to the pure nitrogen treatment; otherwise tissue breakdown will occur even when the above precautions are closely followed. Tissue breakdown did not occur in the presence of oxygen. In preliminary experiments it was found that even the small amount of oxygen present in unscrubbed nitrogen was sufficient to prevent the breakdown of the potato tissue during storage.

RESULTS

Oxygen supply and breaking dormancy. As shown in Table I, dormant potatoes sprout very readily when held in 5 to 10 per cent of oxygen under a moist condition, or in 2 per cent of oxygen under a relatively dry condition, but the dormant potatoes do not sprout when held in 20 per cent or higher percentages of oxygen. In fact, the potatoes held in the relatively dry condition do not germinate in as much as 10 per cent of oxygen. This germination of the potatoes in 12 days in a reduced percentage of oxygen is exceedingly rapid when compared with a requirement of 54 days of

TABLE I

SPROUTING OF IRISH COBBLER POTATOES* DURING EXPOSURE TO VARIOUS CONCENTRATIONS OF OXYGEN AT 23° TO 28° C.

First treatment		Number of whole tubers sprouting** out of 6 in each treatment											
		Days held in first treatment started approximately 10 days after harvest						Removed from first treatment and held for additional days					
		Moist			Dry			Moist in 5% O_2			Dry in 2% O_2		
% O_2	% N_2	12	18	23	12	18	23	5	15	21	5	15	21
0	100	0	2	3	0	1	5	—	—	—	—	—	—
2	98	0	2	3	1	5	6	—	—	—	—	—	—
5	95	1	4	6	0	1	5	—	—	—	—	—	—
10	90	2	5	6	0	0	0	—	—	—	2	6	6
20	80	0	0	0	0	0	0	2	4	6	0	6	6
40	60	0	0	0	0	0	0	2	4	5	0	6	6
80	20	0	0	0	0	0	0	0	3	4	0	5	6
100	0	0	0	0	0	0	0	0	3	4	0	4	6

* Harvested in South Carolina.

** Visible sprout at eye of intact tuber.

dry storage in 20 per cent of oxygen for the completion of the usual dormant period and the production of visible sprouts at the eye of intact tubers.

The results of many tests have shown that 2 per cent of oxygen is the optimum concentration for the production of sprouts on freshly-harvested potatoes held in a relatively dry condition. This result is shown by the data in columns 6, 7, and 8 in Table I. In this case good germination was obtained in 2 per cent of oxygen and a reduced rate of germination was obtained in both 0 and 5 per cent of oxygen. Further tests, in which 7 per cent and higher percentages of oxygen were used, showed that these concentrations did not hasten the germination of freshly-harvested potatoes during the storage period of 23 days.

The results of the tests with relatively dry potatoes were in general duplicated with potatoes held in a moist condition. The optimum concentration for germination of dormant potatoes was 7 to 10 per cent, and 12 per cent of oxygen was found by additional tests to be the upper limit for germination and, even then, the potatoes sprouted at a reduced rate. The rate of sprouting of tubers in 12 per cent of oxygen in these additional tests was comparable with the rate shown for 2 per cent of oxygen in Table I, columns 3, 4, and 5. The beginning of germination of the potatoes in 10 and 12 per cent of oxygen was respectively 12 and 18 days from the start of the experiment. At this same time there was no evidence of sprouting of potatoes held in 15, 17, or 20 per cent of oxygen. Upon continuing the experiment it was found that 25 per cent of the tubers in 15 and 17 per cent of oxygen produced the first sprouts on the 23rd day while none of the tubers in 20 per cent or higher percentages of oxygen were sprouting at this time.

Dormant potatoes germinating rapidly in reduced percentages of oxygen (2 to 10 per cent) produce many sprouts per eye in all eyes of the tuber; i.e., apical dominance of the seed end over the stem end, as well as the apical dominance of the top bud over lateral buds of each eye was broken. Three or 4 sprouts, usually 4, can also be obtained from one-eye pieces of tuber held in reduced percentages of oxygen, as compared with one sprout on the one-eye pieces held in a normal percentage of oxygen. A more complete report of this information will be made in a later paper.

Since no sprouting was obtained in the higher percentages of oxygen during the 23 days of treatment, the dormant potatoes were transferred to storage atmosphere containing 5 per cent of oxygen for the moist storage and 2 per cent of oxygen for the relatively dry storage condition. The results of this procedure are shown by the data in Table I, columns 9 to 14. Sprouting of the dormant potatoes took place in the reduced concentrations of oxygen which shows not only that a low per cent and not a high per cent of oxygen was influential in promoting the sprouting of dormant potatoes, but also that storage of the potatoes in high percentages of oxygen had no harmful effect on the buds. Thus the potatoes remained dormant in normal and increased percentages of oxygen, not because of the inability of the buds to grow, but because they obtained too much oxygen.

Although anaerobiosis, caused by storage in the absence of oxygen, has been recommended previously (8) for the breaking of dormancy of freshly-harvested potatoes, the method is not as effective or desirable a procedure as storage in the presence of a low percentage of oxygen. This conclusion is based upon the fact that dormant potatoes sprout at a much slower rate when held in the absence of oxygen than when held relatively dry in 2 per cent or moist in 10 per cent of oxygen as is shown by the data in Table I. Since the presence of oxygen in the storage atmosphere precludes an anaerobic condition other than that which may be thought to occur normally in the cells of the potato tuber even in an abundance of oxygen, one is justified in concluding that the complete absence of oxygen is not desirable for breaking the dormancy of freshly-harvested potatoes. This is especially true when one considers the precautions that must be taken to prevent physiological breakdown of the potato tubers stored in the absence of oxygen. Furthermore, carbon dioxide which is effective in breaking the dormancy of potatoes is usually not eliminated during the anaerobiosis treatments so that the results are complicated by the action of an additional factor. In the present experiments, however, the carbon dioxide was removed as rapidly as produced and eliminated from the tissue.

Sprouting of dormant potatoes will occur somewhat earlier if the tubers are placed in low percentages of oxygen immediately after harvesting than if the tubers have been allowed to dry after being harvested and previous to placing in low partial pressures of oxygen. As shown in Table II, tubers held moist in 5 to 10 per cent of oxygen started to sprout within 7 to 9 days after harvest and those held relatively dry in 2 per cent of oxygen started to sprout within 9 days after harvest. These results are to be compared with 12 days of treatment to produce sprouts on tubers that were allowed to dry for 10 days preceding the treatment. No doubt this slight difference in period of treatment required to produce sprouting is influenced by the difference in rate of respiration of the freshly-harvested and the slightly dried potatoes.

As the potato tuber becomes older, from time of harvest, it will sprout in progressively higher precentages of oxygen when held in either a moist or a relatively dry condition as is shown by the data in Table II. Potato tubers placed under treatment in a moist condition, immediately after harvest, will sprout in 7 days in 10 per cent of oxygen, in 23 days in 20 per cent of oxygen, and in 36 days in 50 to 100 per cent of oxygen. A somewhat similar condition exists with potatoes held in a relatively dry condi-

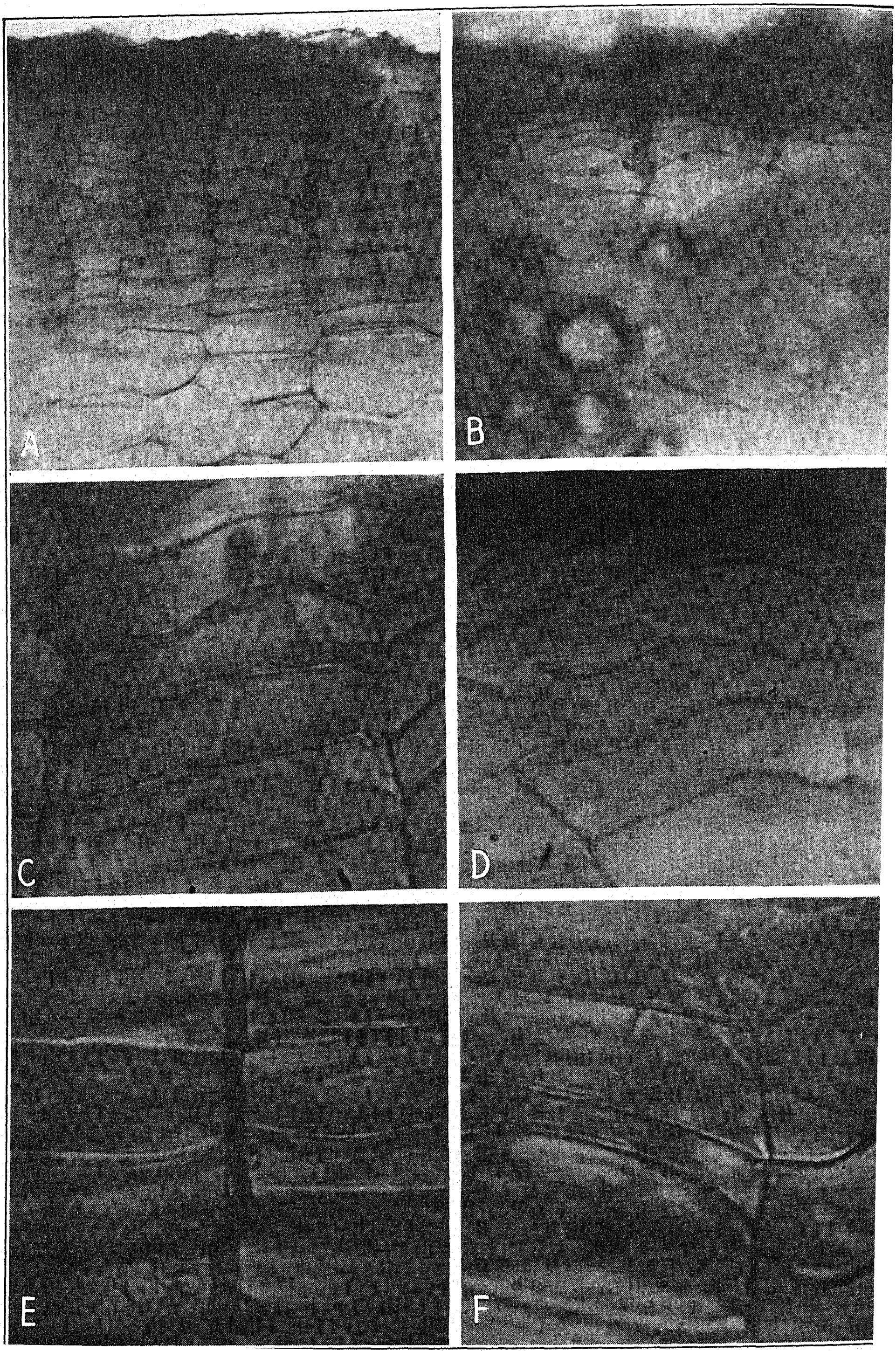
A
B
C
D
E
F

TABLE II

PROGRESSIVE INCREASE IN DAYS REQUIRED FOR SPROUTING OF IRISH COBBLER POTATOES* HELD IN BOTH A MOIST AND A DRY CONDITION IN VARIOUS PERCENTAGES OF OXYGEN AT 23° TO 28° C.

Condition and treatment			Number of whole tubers sprouting** out of 4 in each treatment											
			Days after harvest											
	% O_2	% N_2	7	9	12	15	23	30	34	36	40	43	47	50
Stored in moist moss	5	95	0	2	3	4								
	10	90	1	3	4									
	20	80	0	0	0	0	1	3	4					
	50	50	0	0	0	0	0	0	0	2	4			
	100	0	0	0	0	0	0	0	0	1	2	3	4	
	Room		0	0	0	0	0	4						
Stored dry	2	98	0	1	4									
	5	95	0	1	1	1	4							
	10	90	0	0	0	0	0	0	2	2	3	4		
	20	80	0	0	0	0	0	0	0	0	1	2	4	
	50	50	0	0	0	0	0	0	0	0	0	3	4	
	100	0	0	0	0	0	0	0	0	1	3	4		
	Room		0	0	0	0	0	0	0	0	0	0	2	4

* From Institute garden.
** Visible sprout at eye of intact tuber.

tion during treatment with various percentages of oxygen as is shown in the lower part of Table II. Storage of potatoes in 2 per cent of oxygen in relatively dry condition brought about early sprouting while 10 to 100 per cent of oxygen caused considerable delay in the production of sprouts.

Anatomical study of the periderm. Since it appeared that the reason for the sprouting of the potato tubers was to be found in the rate of thickening of the periderm under various conditions, microscopical study of the anatomy of the potato tuber, especially of the skin or periderm layer surrounding the tuber, was undertaken. It was observed that the rate of formation of the periderm depends mainly upon the external conditions, moist conditions hastening and dry conditions retarding its formation. As shown by Figure 1 A, the periderm of the dormant tuber held in moist storage (in experiment in Table II) for 16 days increased in depth from 2 to 4 layers of cells at the time of harvest to 9 to 12, usually 10 layers of

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FIGURE 1. Photomicrographs showing the periderm of Irish Cobbler potato tubers held in 20 per cent of oxygen for various periods under different moisture conditions at 23° to 28° C. The periderm contains many more layers of cells after the tuber has been held (A) 16 days in moist moss, than it does when held (B) 16 days in a dry condition. Also the moist condition is more favorable than the dry condition for the formation of a thick cell wall and the cell walls become thicker with an increase in the period of storage as shown in (C) 16 days in moist moss, (D) 16 days in dry condition, (E) 36 days in moist condition, and (F) 36 days in dry condition. (Magnification A and B, ×180; C, D, E, and F, ×730.)

rectangular cells, arranged with their longer axes parallel to the surface. Most of these cells contain a nucleus. In addition to these cells there is the cork region which consists of from 1 to 4, usually 2 or more, layers of empty collapsed cells. These empty cells appear compact with the thickened walls touching one another as a result of the loss of the cell contents. At the end of the same period of storage the periderm of tubers held in a relatively dry condition was much thinner (Fig. 1 B), consisting of from 4 to 6 layers of rectangular cells with the cells of the inner 2 to 3 layers containing a nucleus. The corky tissue consisted of 1 to 2 layers of collapsed cells. The average thickness of the periderm tissue under these two conditions was 0.25 mm. for the moist-held and 0.07 mm. for the relatively dry-held tubers. As the storage period was continued the number of living rectangular cells in the periderm did not increase in the potatoes under moist and dry conditions, but there was a gradual thickening of the cell walls accompanied by an accumulation of collapsed cells at the surface of the tubers.

A more detailed examination of the periderm showed also that the cell walls were thicker in the potatoes held under moist conditions than in those held under a relatively dry condition for 16 days. The photomicrographs in Figure 1, C and D, show the relative thickness of the cell walls of the periderm of potatoes from the respective conditions. As the storage period was increased to 36 days the difference in thickness of the cell walls became even more striking as shown in Figure 1, E and F.

When the potato tuber was examined microchemically for suberin by staining with Sudan III, very little suberin was found in the periderm of the freshly-harvested tuber, but it increased as the storage period was extended. The greatest amount of suberin was found in the cell walls just at and under the surface of the tuber, and the least was found in walls of the innermost cells of the periderm. As the cell walls thickened, especially under the moist condition, there was observed a greater abundance of suberin in the membranes. By comparative observations it was estimated that there was much more suberin present in the cell walls of tubers held in a moist condition than in the cell walls of tubers held in a relatively dry condition. It is apparent that the deposit of suberin in the cell walls, the thickening of the cell walls, and the increase in the number of cells decrease the permeability of the skin of the potato to oxygen so that as the potato tuber becomes older it will germinate in increasingly higher percentages of oxygen. This is especially true in the cases in which the tubers are held under a moist condition. Microscopical examination showed the cell walls to be thicker at the time of sprouting in 100 per cent than in 20 per cent of oxygen in the moist condition. In the relatively dry condition, however, there was no observable difference in the thickness of the cell walls of the periderm of the tubers at the time of sprouting in 10 and 100 per cent of oxygen.

The data in Table II combined with the microscopical observations lead to the conclusion that the moisture conditions of the storage atmosphere are exceedingly important in this study of the rôle that oxygen plays in the dormancy of the potato. As shown in Table II the potatoes held in a normal amount of oxygen (20 per cent) sprouted in 23 days when held moist in a container, in 40 days when held relatively dry in a container, and in 47 days when held dry in a room at 23° to 28° C. Thus we find that a comparatively short period of time is required under a moist condition to bring about an increase in thickness of the periderm sufficient to reduce the passage of oxygen into the tuber so that the internal concentration would be comparable with that present when the freshly-harvested tubers are exposed to an atmosphere containing 10 per cent of oxygen. Likewise, the tubers stored under the dry conditions require a longer period of time to attain the same end because the rate of suberization is so much slower. The suberized tissue that is formed under relatively dry conditions does not allow for an easy penetration of oxygen even when the diffusion gradient is exceedingly high because of the compactness and "insulating effect" of the many layers of empty cells that are relatively dry. Thus the tubers held in a dry condition will produce sprouts at approximately the same time in 10 and 100 per cent of oxygen. When this tissue is kept moist under the same conditions some oxygen will diffuse through the tissue. And when the cell walls become thicker and more numerous under the moist conditions the potatoes will sprout as readily as those held under dry conditions when exposed to 100 per cent of oxygen. This explains why the potatoes held in 100 per cent of oxygen in both a moist and a dry condition sprouted at the same time, as shown by the data in Table II.

Temperature is another important factor indirectly regulating the passage of oxygen into the freshly-harvested potato tubers. High temperatures increase the rate of metabolic activity of the potato tissue thereby increasing the rate of suberization while low temperatures have the opposite effect. Storage at low temperature does not quickly shorten the period of dormancy of the potato, as it does in many types of plant tissue, because it retards metabolic activity which in turn retards the formation of the periderm thereby allowing the penetration of too much oxygen into the potato tissue. After prolonged storage at low temperature the dormancy of the potato is overcome because of two reasons: a slow thickening of the periderm during storage, and a very decided increase in the metabolic rate upon removal to higher temperature at the end of the period of storage under which conditions the periderm does not allow for the passage of sufficient oxygen. It is also possible that the periderm can thicken (at a greatly reduced rate) sufficiently at the low temperature, if the tubers are kept there long enough to bring about a reduction in the penetration of oxygen to a sufficiently low level that sprouting will take place.

Varietal differences in the rate of formation of the periderm. Microscopical examination of the varieties Irish Cobbler, Bliss Triumph, and Golden leads to the conclusion that potatoes that naturally have a short rest period, or period of dormancy, develop the thickened periderm more rapidly than potatoes that have a long period of dormancy. Ten days after harvest the Irish Cobbler tubers had 3 intact and 1 empty cell in the periderm while the Golden variety had from 5 to 6 intact cells and usually 3 empty cells in the periderm. The Golden variety was sprouting at this time while the Irish Cobbler variety did not sprout until many weeks later. At this same time the periderm of the Bliss Triumph variety was only slightly thicker than that of the Irish Cobbler. Another lot of Irish Cobbler potatoes (harvested 3 months earlier in South Carolina and sprouting at the time of examination) was examined and it was found that the periderm consisted of 8 intact cells and 4 to 6 empty cells at the surface of the tuber. All of these cells had greatly thickened cell walls. Thus the rate of formation of the periderm layer determines to a great extent the duration of the dormant period of the potato tuber.

Immature potatoes are considered more dormant than mature tubers and here again the periderm plays the important rôle in regulating the penetration of oxygen into the tuber. Immature tubers are under-developed and require an additional period of time to develop a thickened periderm and to block effectively the lenticels in order to retard the passage of oxygen while the mature tubers at the time of harvest have completed the early processes of suberization. Thus the immature tubers require a longer period of rest because they are not as far advanced in the formation of the periderm that retards the penetration of oxygen as in the case of the more mature tubers.

Sprouting of non-dormant potatoes in various percentages of oxygen. High percentages of oxygen do not prohibit the sprouting of potato tubers when the periderm has become sufficiently thick to inhibit the passage of oxygen into the potato tuber. Irish Cobbler potatoes held for 36 or more days (Table II) in 50 and 100 per cent of oxygen began to sprout at this time and produced good growth while still exposed to the treatment. In still another experiment some potatoes of the variety Golden, which has a relatively short period of dormancy, were placed in 10, 20, and 50 per cent of oxygen at the time when sprouts first began to appear. At the end of 12 days of treatment it was found, as shown in Figure 2 A, that tubers held moist in 10 per cent of oxygen sprouted to a greater extent than tubers held in higher percentages of oxygen. The tubers held in 20 per cent of oxygen were the next to sprout while the tubers in 50 per cent of oxygen showed little or no additional growth of the sprouts. Continuing the treatment for 21 days produced considerably more growth of the sprouts in 10 and 20 per cent of oxygen and especially in 50 per cent of oxygen (Fig. 2 B). Results comparable to these were obtained also with potatoes held in a relatively dry condition during the period of treatment with various percentages of oxygen.

Since all the tubers taken from dry storage were sprouting to a slight extent at the time this experiment was started it was expected that the tubers held dry in 10 and 20 per cent of oxygen would continue to sprout at the same rate while those in 50 per cent of oxygen would be retarded in sprouting until such time as the periderm would thicken to retard the passage of oxygen. When these tubers were placed under a moist condition there was to be expected a temporary increase in the penetration of oxygen so that sprouting would be somewhat retarded by 20 per cent and greatly retarded by 50 per cent of oxygen. The results of the tests conducted for 20 days show that the tubers sprouted as expected. It is apparent that the tubers sprouted more rapidly in 10 per cent of oxygen under the moist condition because the diffusion gradient between the external and internal

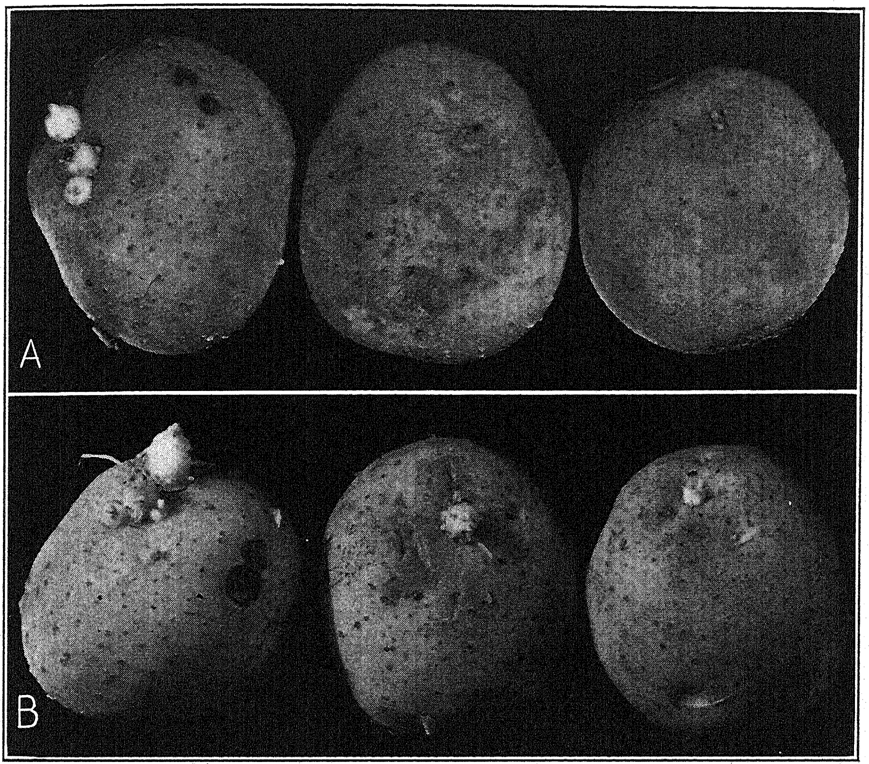

FIGURE 2. Sprouting of non-dormant potatoes placed in, left to right, 10, 20, and 50 per cent of oxygen in a moist condition at 23° to 28° C. for (A) 12 days, (B) 21 days. (Photograph ×0.7.)

atmosphere of the tuber was not very great; thus only sufficient oxygen needed to promote sprouting penetrated the moist tissue. In 20 and 50 per cent of oxygen the moist tubers were exposed to a steeper diffusion gradient and sprouting was retarded until the periderm had thickened sufficiently to inhibit the passage of oxygen into the tuber. Similar results were likewise true for the tubers held in a relatively dry condition, except that tubers in 10 and 20 per cent of oxygen sprouted at about the same rate, indicating that suberization had taken place to a considerable extent in these tubers. However, when the tubers were held in 50 per cent of oxygen, suberization had not taken place to a sufficient extent to prevent the intake of a large amount of oxygen, thus sprouting was delayed. When the periderm had thickened sufficiently, sprouting of these tubers in 50 per cent of oxygen in both a moist and a dry condition took place at a rate as rapid as that of tubers stored in a normal concentration of oxygen.

Sprouting of cut potatoes in various concentrations of oxygen. One-eye pieces of partially dormant (31 days after harvesting) potato tubers, cut so that there would be a representative eye from all portions of the tuber in each treatment, were exposed to various concentrations of oxygen in both a moist and a relatively dry condition. The earliest sprouting occurred within four days when it was observed that 30 per cent of the pieces held in a dry condition in 5 per cent of oxygen were sprouting. On the seventh day of the treatment, 20 per cent of the pieces were sprouting in 2 per cent of oxygen and on the eleventh day 40 and 10 per cent of the pieces were sprouting in 10 and 20 per cent of oxygen respectively. Sprouting of 20 per cent of the one-eye pieces in 80 per cent of oxygen did not occur until after 21 days of treatment in the dry condition. Whenever sprouting started in any treatment it was observed that complete sprouting of all the one-eye pieces in that treatment took place at a rapid rate.

One-eye pieces of potato tubers held moist in various concentrations of oxygen started sprouting slightly later than the one-eye pieces held in a relatively dry condition. Seventy per cent of the pieces in 10 per cent of oxygen and 20 per cent of the pieces in 20 per cent of oxygen produced sprouts within 7 days after the start of the treatment. Also 30 per cent of the tubers in 80 per cent of oxygen showed sprouts within 11 days of the treatment.

When non-dormant yet not noticeably sprouting potatoes were cut into one-eye pieces and exposed to various concentrations of oxygen there was observed high percentage of sprouting in all concentrations of oxygen up to and including 20 per cent. The earliest show of sprouts and the best growth of sprouts during the treatment was observed with the low percentages of oxygen in both the moist and relatively dry conditions as have already been discussed. Germination, however, was greatly retarded with concentrations of oxygen above 20 per cent by volume and in every case the retardation was in proportion to the increase in per cent of oxygen. Pieces of tubers held moist in 7 per cent of oxygen required 3 days for a show of sprouts while those in 80 per cent of oxygen required 14 days. Likewise, pieces of tubers in relatively dry condition required 5 days for a show of sprouts in 2 per cent of oxygen and 19 days to produce sprouts in 80 per cent of oxygen. These results show that the sprouting of the cut potato tubers depends upon the rapidity of the formation of wound periderm and the deposit of suberin in the cell walls of both the old and new surfaces of the cut tuber.

The results of these experiments with partially-dormant and non-dormant potato tubers cut into pieces and subjected to various percentages of oxygen show the importance of the formation of the wound cork layer in the production of sprouts. In every case, whether dormant or non-dormant, the one-eye pieces sprouted relatively quickly in low percentages of oxygen, but were greatly delayed in sprouting when high percentages of oxygen were present. Likewise those pieces in a moist condition sprouted earlier in high percentages of oxygen than the pieces held in a relatively dry condition because the moist condition is more conducive to the rapid development of the wound cork tissue as well as to further suberization of the original periderm. This situation with the cut tubers is different from that found with whole intact tubers where only the normally-formed periderm is involved. The periderm of intact tubers forms more slowly and never to the same extent as wound periderm. Thus the penetration of oxygen into wounded tubers is quickly retarded while it is only slowly retarded in the case of the whole tubers. This accounts for the fact that sprouting of whole tubers was greatly retarded by high percentages of oxygen as shown in Table II.

Shortening the rest period by cutting or peeling the tubers. Appleman (1) reported that the rest period of freshly-harvested potatoes could be greatly shortened by cutting the tubers into pieces or by removing the skin and holding the pieces in a moist condition while exposed to the normal oxygen content of the air. Also he found that whole tubers with skins removed could be sprouted within 10 days after harvest if the stem end of the tuber was placed in soil and two-thirds of the tuber was covered with 2 to 3 inches of moist excelsior in order to keep the tuber moist as well as to expose the buds to the maximum partial oxygen pressure of the atmosphere. Appleman's results were confirmed in these experiments by many tests carried out in numerous ways with both Irish Cobbler and Bliss Triumph varieties of potatoes. But it is believed that cutting the tuber hastens sprouting not by increasing the passage of oxygen as suggested by Appleman but by decreasing it. Shortly after cutting the tuber there occurs a deposit of suberin at the cut surface which effectively retards the loss of water and the passage of oxygen. Within a short time there is

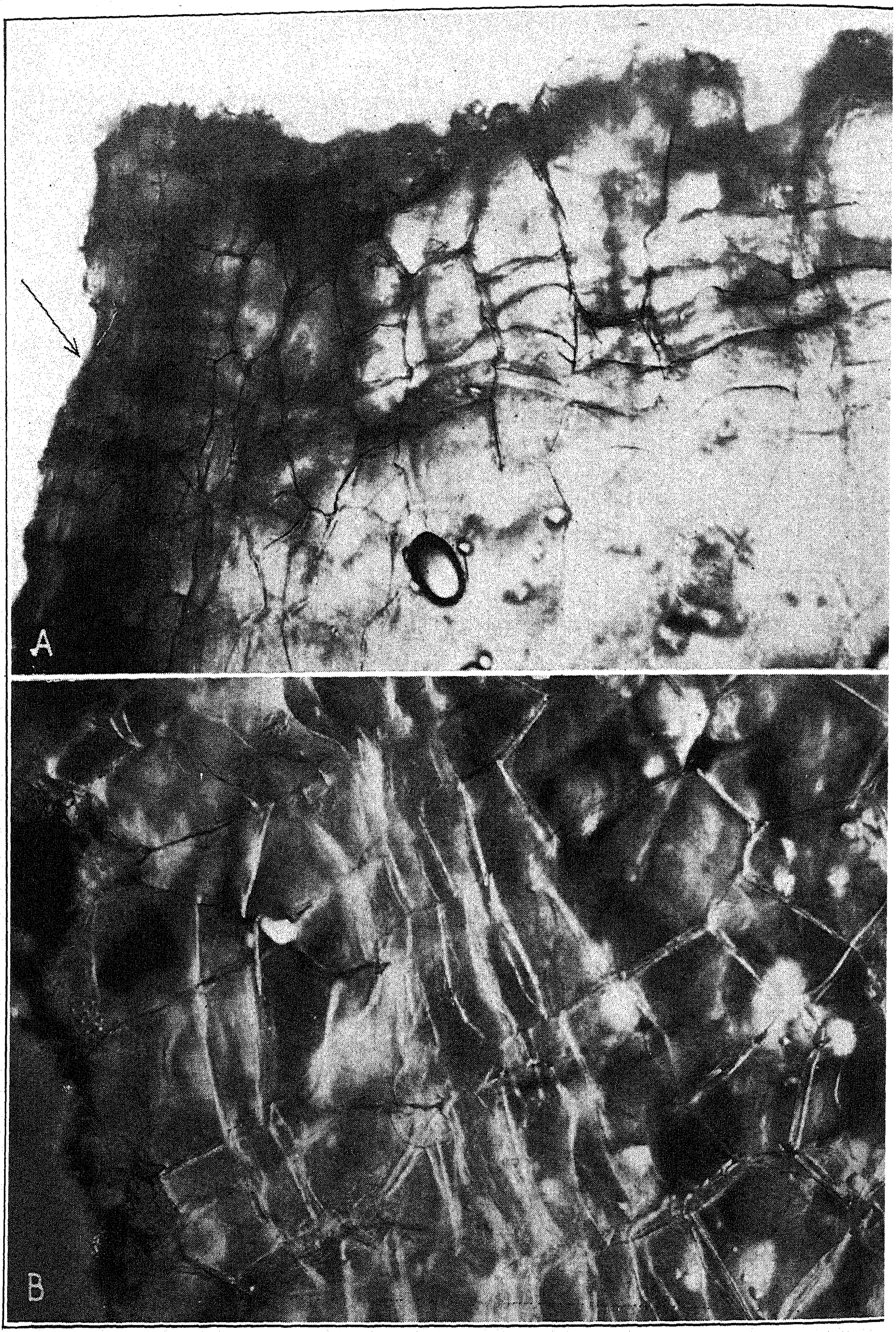

FIGURE 3. (For description see legend on opposite page.)

formed the new periderm or "wound cork" tissue which rapidly becomes deeper and the cell walls thicken very markedly. This is especially true at the soil and air temperatures usually used for sprouting of these pieces of tubers, as Artschwager (2) and Smith (10) have shown that high temperatures favor and low temperatures retard suberization of wounded potato tubers. Figure 3 A shows a section of this wound periderm (top, right) produced in a one-eye piece of tuber 8 days after cutting from the parent potato and planting in the soil. Also is shown in this photomicrograph the normal periderm tissue (left edge) containing approximately 8 rectangular cells, much of which developed after placing in the moist condition, for comparison with the wounded tissue which contains a few rectangular cells in addition to the thickly suberized wound-surface tissue. Usually within 2 weeks in soil this new wound periderm tissue is approximately 0.45 mm. deep as compared to 0.25 mm.—the depth of the original periderm of the tuber—and it presents an effective barrier to the penetration of oxygen. The relative thickness of this "wound cork" tissue at the time of sprouting as compared with the original periderm of the potato is shown in Figure 3 B. Thus we see that cut, or wounded, potato tubers sprout faster than whole, or uncut, potato tubers because of the differences in the rate of formation of the periderm layer. When the permeability of the tissue to oxygen is quickly limited by suberin and "wound cork" formation, rapid sprouting of the tuber or piece of tuber will take place. However, if the tuber is not injured suberization takes place slowly, in a normal manner, and the permeability of the tissue to oxygen is not reduced sufficiently to permit sprouting until 6 or 8 weeks or more after harvest.

Effect of frequent removal of the periderm on sprouting of the potato. If wounding hastens germination because the oxygen supply is restricted by the new periderm that is formed after cutting, then the frequent removal of the external layer should prevent this corky membrane from forming and sprouting should be retarded rather than favored by cutting the tuber. This was tested with one-eye pieces of dormant Irish Cobbler potatoes that had been held for 31 days at 24° C. after harvest. The pieces were planted in soil in four distinct lots. One lot was not treated, but it was removed from the soil for observation along with the other three lots which were dug up every second day for further treatment. This treatment was carried out as follows: the skin about the eye of the second lot was not disturbed but the original cut surface was peeled; the skin about the eye of the third lot was removed but the original cut surface was not disturbed;

FIGURE 3. Photomicrographs of sections of a piece of potato tuber showing (A) "wound cork" formation with a few cross walls forming the rectangular cells of the new periderm 8 days after cutting. The original skin or periderm is indicated by the arrow. (B) Section showing the "wound cork" tissue with many cross walls in the large cells, 16 days after cutting the surface shown at the left edge. (Magnification of A and B, ×190.)

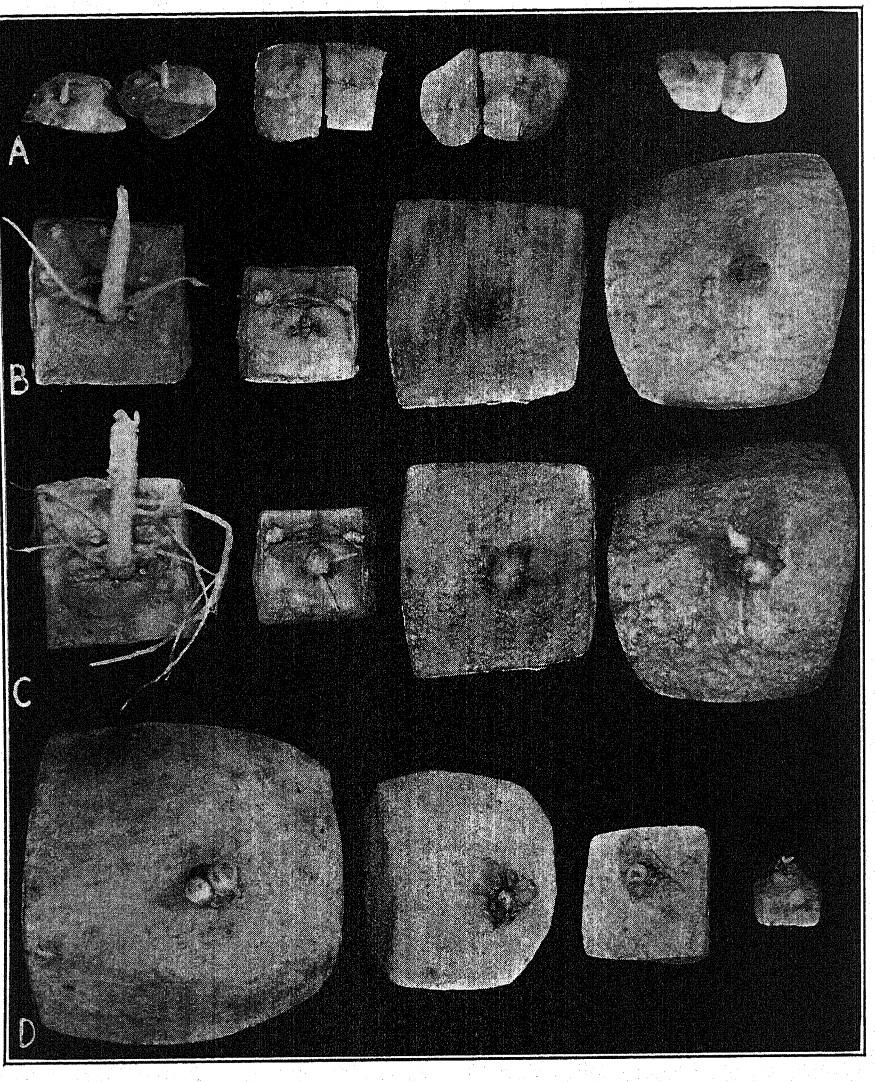

FIGURE 4. The sprouting of one-eye pieces of partially dormant potatoes was retarded by facilitating the entrance of oxygen by repeated cutting of the surface of the tuber. Comparative samples of Irish Cobbler (first row) and Bliss Triumph (second row) were used in each treatment as follows (left to right): (1) control, not cut; (2) skin around eye left intact, but cut surface peeled; (3) skin peeled from around eye, but the original cut surface not disturbed; and (4) all surfaces peeled. Photographed, 0.9 natural size, after (A) 10 days, and (B) 18 days of treatment. Sprouting in lots 2, 3, and 4 was retarded until the "wound cork' tissue was allowed to form. The cutting of (B) tubers was stopped after 18 days and (C) shows bud growth on these pieces of tubers by the 28th day; (D) shows that size of tuber-piece did not materially influence the *time* at which spouts were produced.

and the fourth lot was completely peeled. Sprouting was first observed at the end of 8 days in the untreated or check lot and these sprouts continued to develop during the period of the test. Two days later sprouts were showing on the second lot which was treated every 48 hours by having the sides peeled. At the end of 14 days the seed pieces in the untreated or first lot had sprouts 10 mm. long, those in the second lot had sprouts 5 mm. long, and those pieces in lots 3 and 4 were not producing sprouts. A representative sample of seed pieces from each of the four treatments at the end of 10 days is shown in Figure 4 A. In another lot of partially dormant potatoes of the Bliss Triumph variety, the surface of the pieces was cut every 24 hours for 18 days with the result, as shown in Figure 4 B, that the control pieces sprouted while the treated pieces were prevented from sprouting. Thus we see that cutting away the surface of the tuber as it suberizes allows for an easy entry of oxygen and the bud does not grow. According to Artschwager's (2) observations, which were confirmed in these tests, this suberized layer would have to be removed within every 48 hours to make certain that an effective layer did not form. Whenever this cut surface is undisturbed for a sufficient period of time to allow for the development of the "wound cork" tissue about the eye, sprouting will take place. This eventually happens to all of the cut tubers as it is impossible to continue indefinitely to cut away the suberized tissue about the eye without causing injury which would then prevent the bud from growing. In no case did the buds on the tubers that were cut frequently fail to grow when the surface was allowed to suberize, as shown in Figure 4 C. If non-dormant potatoes (determined by a show of sprouts on a few of the tubers in storage) are used for these tests the results are somewhat complicated since the buds have already been exposed to low partial pressure of oxygen and their growth has been initiated. However, tests have shown that this growth, although initiated, can be greatly retarded by the procedure already outlined. With the non-dormant seed pieces, sprouting was first observed in the first or untreated lot and at the end of one week the longest sprouts were observed on the pieces in this lot. The seed pieces in the second lot were the next to produce sprouts which were slowly followed by the seed pieces in lots 3 and 4. At the end of 10 days the average length of sprouts on pieces of tubers in each treatment was as follows: 20 mm. in lot 1, 10 mm. in lot 2, 3 mm. in lot 3, and 1 mm. in lot 4. Growth of the sprouts in lots 3 and 4 progressed very slowly as long as the wound periderm was being removed, and as soon as it was allowed to form for a few days the sprouts proceeded to grow at a rate comparable with the first or untreated lot of seed pieces.

Frequent removal of a thin slice from the surface of the cut tuber reduces to some extent the size of the piece that is left to produce a sprout. However, the reduction in the size of the seed-piece by this process of frequent removal of a thin slice was not a factor in the delay in sprouting. This was shown by the following tests: (a) no difference was observed in the rate or order of emergence of sprout in seed-pieces which varied in size from 15 mm. to 40 mm. on a side; (b) at the start of the experiment the size of the seed pieces was so adjusted that the slices that were removed provided for reaching the end of the test with seed-pieces of the same size. In one test conducted according to (b) the suberized tissue was cut away every 48 hours with the result that sprouting was retarded for a period of 18 days at which time the size of the seed-piece (from a dormant tuber and held in a moist chamber) was reduced to approximately 8 mm. on a side. When removal of slices was discontinued and the suberized layer was allowed to form for a few days, the buds began to grow as quickly on the smallest pieces as on the largest pieces of potato (Fig. 4 D), and the only apparent difference in these buds was in their size which varied directly with the size of the seed piece.

Effectiveness of periderm at eye tissue and inter eye tissue on growth of the bud. The results of the cutting or peeling tests indicate that the periderm adjacent to the eye of the potato tuber is more important in regulating the dormancy of the buds than the periderm formed some little distance from the eye. This is indicated by the fact that the pieces of tubers that had the cut surfaces recut frequently while the normal periderm around the eye was undisturbed, germinated almost as rapidly as the pieces of tubers that were not recut during the test. When, however, the periderm was removed from about the eye there occurred a great delay in the production of sprouts because of the easy entry of oxygen into the tissue adjacent to the buds. Likewise, when pieces of tubers are peeled once and then allowed to form a "wound cork" tissue over the peeled area about the eye tissue, sprouting will occur much faster than with pieces that were not peeled. And peeling, cutting, or removing a portion of the tissue from about an eye of the tuber will promote the growth of the buds in that particular eye in advance of the growth of buds in the other eyes of the potato tuber, irrespective of the position of this eye in relation to the apical or topmost eye of the tuber. Thus it is possible that the rate of metabolism of the tissue adjacent to the eye and the thickness of the periderm over this area controls the amount of oxygen available to this tissue which in turn regulates the mechanism that promotes or retards the growth of the buds.

DISCUSSION

Appleman (1) stressed the necessity of keeping freshly-harvested potatoes moist to retard the suberization of the skin which he believed led to a prolongation of dormancy by preventing the passage of oxygen into the potato tuber. Finding that this moist treatment led to an early production of sprouts on dormant tubers, he then concluded that a maximum partial oxygen pressure of the atmosphere was necessary for the breaking of the dormancy of potato tubers. He found in experimenting with peeled tubers, tubers cut into one-eye pieces and held in a moist condition, covering the cut surface of the pieces with paraffin to prevent evaporation, or with whole tubers wrapped in cotton saturated with hydrogen peroxide, that early sprouting was obtained because, he believed, the treatments had facilitated or provided for the entrance of oxygen. As shown by the results presented in this paper, all of these experiments conducted by Appleman would produce a greater rate of periderm formation or suberization of the tubers, rather than reduce it, which would quickly retard the entrance of oxygen. The treatment of the tubers with hydrogen peroxide was effective in shortening the rest period because the tuber was exposed to excellent conditions for suberization and not because the chemical was effective in providing for an increased supply of oxygen to the tuber when it was broken down by the catalase present in the potato.

The results of the present investigation indicate that any condition that tends to hasten the formation of the periderm will shorten the dormant period of freshly-harvested potato tubers. In fact, under some conditions the new tubers could sprout and produce a new plant while the mother plant continues to grow in the soil. Appleman (1, Fig. 17, p. 220) showed this to take place in the greenhouse and he believed the second growth to be brought about by good aeration of the tubers by keeping them moist and protected from the sun. The results of the present experiments, however, indicate that these tubers sprouted because the moist conditions at midsummer temperatures promoted the rapid formation of the thickened periderm which retarded the penetration of oxygen into the tissue.

Smith (9) likewise considered that high percentages of oxygen may bring about the elimination of dormancy in the potato tubers since tubers in 60 per cent of oxygen absorbed more of this gas than those held in air. He reported that there was "a large oxygen absorption by the tubers subjected to those treatments and conditions which shorten the rest period." Thus he believed that the permeability of the potato tuber to oxygen was increased with holding in a moist condition, and with treatment with chemicals. His interpretation of these data was that the moist stored lots became increasingly permeable to carbon dioxide during the storage period. An examination of the data in his tables on composition of internal gas of the non-dormant tubers (102 days after harvest) just previous to sprouting shows that the oxygen content was found to be 7.0 to 9.7 per cent for the moist-held tubers and 13.0 to 13.9 per cent for the dry-held tubers, which is considerably below the percentage of oxygen present in the external atmosphere of the potato tuber. In accordance with these data, Davis (5) found that the internal concentration of oxygen in Rural

New Yorker potatoes was as low as 10 to 11 per cent nine weeks after harvesting, thus indicating that the permeability of these tubers to oxygen must have been greatly reduced during dry storage.

In contrast to these results Rakitin and Suvorov (8) have considered temporary anaerobiosis necessary for the production of sprouts on dormant potatoes. They concluded that anaerobiosis (resulting in the production of alcohol and acetaldehyde in the tissue) was the factor in causing early sprouting of the tubers stored for 168 hours in ether, carbon dioxide, or hydrogen. They completely overlooked the stimulating effect that ether would have on the potato tuber and that it would be effective in breaking the dormancy of the potatoes either in the presence or absence of oxygen. Likewise they totally disregarded the action of either pure carbon dioxide or that produced by the tuber in ether or hydrogen, on the production of sprouts by the dormant potatoes. Since Rakitin and Suvorov did not vary their experimental procedure to study the effectiveness of ether, carbon dioxide, or hydrogen with zero and increased percentages of oxygen or make gas analyses of the storage atmosphere, they can only assume that they were dealing with anaerobiosis. It has been shown by the writer (13) that carbon dioxide will bring about the sprouting of dormant potatoes regardless of whether it is present in low or high concentrations and whether oxygen is present simultaneously in low or high concentrations or entirely absent from the storage chamber.

Weber (14) considered the effectiveness of acetylene, nitrogen, hydrogen, and carbon dioxide in shortening the rest period of buds of the lilac (*Syringa vulgaris* L.) as due to the exposure of the plant to an atmosphere free of oxygen. But he did not make measurements of the oxygen present in the storage atmosphere and he overlooked the direct effect of acetylene and of the accumulating carbon dioxide on the dormancy of the buds.

Boresch (3, 4), experimenting with trees and shrubs in warm water bath, in air baths at normal and reduced atmospheric pressure, and in increased oxygen pressure at 30° C., concluded that the dormancy of the buds was overcome as a result of exposure to a condition of high rate of respiration in a restricted supply of oxygen which led to the formation of alcohol and acetaldehyde, and that these substances were responsible for the breaking of dormancy. He exposed lilac twigs to a partial vacuum (50 mm. Hg) which reduced the oxygen content, according to his data, to 0.5 per cent by volume at the start of the experiment. During the course of the experiment, he found it necessary to frequently renew the vacuum in order to maintain the desired pressure and in so doing he believed he was able to remove the respired carbon dioxide. Boresch overlooked an important consideration in this practice; that the oxygen supply was depleted by the respiration of the twigs in less than one-quarter of the period of treatment (even under the poorest conditions of respiration) according to approximate calculations with the aid of data from the report by Denny and Miller (6), so that as the experiment was continued the buds were exposed to an increasing amount of carbon dioxide which he did not eliminate from the experiment. Acetaldehyde is formed as anaerobic respiration takes place and this can, as shown by Miller (7), stimulate the rate of respiration so that continuously a still greater quantity of carbon dioxide is formed. Boresch disregarded the possible effect of the carbon dioxide on the dormancy of the buds since he believed acetaldehyde to be the active agent in shortening the rest period. As has been shown (12, 13) carbon dioxide is effective in shortening the rest period of buds and it is possible that this gas was an important factor in the experiments of Boresch. Also it has been shown in this paper that a storage atmosphere lacking in oxygen, as desired by Boresch, is not as good a condition for breaking the dormancy as a storage atmosphere containing at least a low concentration of oxygen.

In the present report, any possible effect of carbon dioxide or other gases accompanying oxygen has been obviated, and the direct effect of oxygen alone in various concentrations has been measured. As a result it has been found that a low partial pressure, and not a high partial pressure of oxygen, as has been assumed by one group of investigators, is needed for the sprouting of dormant potatoes; and that a constantly maintained low partial pressure of oxygen is decidedly more effective than the complete absence of oxygen as had been assumed by another group. Thus by a direct approach, the problem of the nature and mechanics of the oxygen effect upon dormancy in the potato tuber has been explained on a basis that is contrary to present theory as shown in reviews in scientific reports and in textbooks of botany and plant physiology.

SUMMARY

1. Freshly-harvested potatoes will sprout in 7 days if held in 5 to 10 per cent of oxygen under a moist condition, in 9 days if held in 2 per cent of oxygen under a dry condition, whereas sprouting of the potatoes will not take place until 47 days after harvest if the tubers are held in 20 per cent of oxygen.

2. Normal, 20 per cent, and higher percentages of oxygen are unfavorable for the production of sprouts on dormant potatoes because the bud tissue is exposed to an excessive amount of oxygen.

3. The skin, or periderm, of the potato tuber is more permeable to oxygen at the time of harvest than at any other period in the life of the tuber and the tubers do not sprout at that time because the bud tissue is furnished too much oxygen.

4. Moist conditions facilitate, and dry conditions retard, the formation of the periderm. This is the reason for the fact that potatoes stored under

moist conditions will sprout earlier than potatoes stored under a dry condition.

5. Upon ageing in storage, the periderm of the potato tuber develops many layers of cells, the cell walls become increasingly thicker, and a greater preponderance of suberin is found in the cell walls.

6. Because of the gradual thickening of the periderm the potato tuber will sprout in progressively higher percentages of oxygen until sprouting is obtained in as much as 100 per cent of oxygen many weeks after harvest.

7. In some varieties of potatoes the periderm thickens more rapidly than in others, thus accounting for the difference in length of natural dormancy with different varieties.

8. The sprouting of non-dormant potatoes can be hastened by storage in 10 per cent of oxygen and temporarily retarded by storage in 50 per cent of oxygen. However, sprouting in 50 per cent of oxygen will occur when the periderm increases in thickness so that it retards the passage of oxygen.

9. The rest period of dormant potatoes can be shortened by removing the skin, because the new periderm or "wound cork" tissue that develops very rapidly is more effective than the normal periderm in retarding the passage of oxygen into the tuber.

10. Cut potatoes placed in high percentages of oxygen are temporarily retarded from producing sprouts until the "wound cork" tissue develops to a greater thickness.

11. The sprouting of one-eye pieces of both dormant and non-dormant potatoes can be prevented by frequently removing the skin or "wound cork" tissue thus permitting the entrance of excess oxygen into the tuber. Sprouting will take place quite rapidly when this tissue is allowed to suberize.

12. The periderm of the tissue adjacent to an eye influences the dormancy of the buds in that eye. The removal of the periderm with subsequent suberization of the tissue hastens the growth of the buds in this eye (because of reduced penetration of oxygen) irrespective of its position in relation to the apical eye of the tuber.

13. Oxygen concentrations of 2 to 10 per cent, which break the dormancy, also cause complete elimination of apical dominance in the buds of an eye as well as in all eyes of the tuber so that each eye produced three to four sprouts instead of one sprout as is usually the case with non-dormant tubers.

14. The experimental results and observations presented in this paper are contrary to the generally accepted theories of the dormancy of freshly-harvested potato tubers. These results show that the dormant potatoes do not sprout because the bud tissue obtains too much oxygen rather than an insufficient supply of oxygen; that the skin of the young tuber is more permeable rather than less permeable to oxygen; that the skin of the tuber

becomes less permeable rather than more permeable as the tuber becomes older; and that peeling or otherwise wounding the dormant tuber brings about a condition that retards rather than facilitates the entrance of oxygen.

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RESPONSES OF PLANTS TO GROWTH SUBSTANCES APPLIED AS SOLUTIONS AND AS VAPORS

P. W. Zimmerman, A. E. Hitchcock, and Frank Wilcoxon

A recently published report (26) showed that two of the crystalline plant growth substances, cinnamic acid and phenylacetic acid, were effective when applied as vapors or solutions. This fact was not surprising, since the molecular constitution of a compound does not necessarily change when the physical condition is changed from the solid to the gaseous state. There was, however, a point of special interest concerning the similarity of plants treated with unsaturated carbon gases (carbon monoxide, acetylene, propylene, and ethylene) and vapors of crystalline growth substances. It has been assumed by several workers that ethylene gas cannot be a growth substance in the same sense that the napththalene, indole, and phenyl compounds are growth substances (14, 21). Thus far, there has been given neither factual evidence nor a satisfactory explanation to support such an assumption. There seems no good reason for placing ethylene in a special category unless it can be shown that its behavior is in some way different from other growth substances. Up to date we know that when the gas is applied, the rate of metabolism is modified, resulting in a quick growth of sensitive tissues, especially the upper side of the leaf. The time required for an evident response to the gas is only an hour or less. Other interesting responses occur within a few days. Similar responses can be induced by applying "accepted" growth substances in a gaseous state. Two of these were mentioned above—that is, cinnamic acid and phenylacetic acid. It now appears that practically all of the growth substances are volatile enough to be applied in the gaseous state.

The purpose of this paper is to report the various responses of plants to volatile growth substances and to show their similarities to responses induced with unsaturated carbon gases and growth substances in solution.

MATERIALS AND METHODS

The following plants were used in the experiments: tomato (*Lycopersicon esculentum* Mill.), corn (*Zea mays* L.), Klondike cosmos (*Cosmos sulphureus* Cav.), marigold (*Tagetes erecta* L.), girasole (*Helianthus tuberosus* L.), *Mimosa pudica* L., *Chenopodium album* L., and garden pea (*Pisum sativum* L. var. Alaska). Of these the tomato constituted the main material.

Three methods were used to apply the vapors. The plants were placed under bell jars or large beakers (2 to 18 liters) with the volatile growth substances in open Petri dishes or watch glasses. The minimum amount of the compounds required to induce the responses was not determined. Generally the amount used was approximately 0.1 gram of crystalline material and one to five drops of substance in the liquid state. The amount disappearing in 24 hours from the open Petri dish was not discernible, and the same samples were used repeatedly for new experiments excepting *cis* cinnamic acid and esters of phenyl compounds.

The less volatile substances were placed with the plants under bell jars which were then partially evacuated. This applied especially to indoleacetic and indolebutyric acids.

Another method was to place the plant under the bell jar or two-liter beaker with the substance in an open Petri dish or watch glass placed on a warm to hot crucible. Partial vacuum was sometimes used though it was not necessary. The temperature used was varied with the volatility of the substance to be tested. For example, *cis* cinnamic acid melts at 42°, 58°, and 68° C. and sublimates at 95° C. or less, depending upon the form (8, p. 344). In this case no heat was necessary but the response could be accelerated when heat was used. With indoleacetic and indolebutyric acids heat and vacuum together were most effective to induce a response of the plant. The esters were effective without or with slight heat. The phenyl esters were the most volatile of all the substances tested, and therefore required no heat to provide vapors.

The time the plants were exposed to the vapors varied from 3 minutes to 24 hours.

Ethylene gas was injected into the bell jar containing the plants. The concentration varied from 1 to 1000 p.p.m. of air.

EXPERIMENTAL RESULTS

RESULTS PREVIOUSLY REPORTED

In order to avoid repetition and save space it is desirable to summarize the previously reported responses of plants induced by unsaturated carbon gases and crystalline growth substances.

The earlier reports dealt with growth substances applied in solutions (9, 27, 24). Under certain conditions local responses were induced where the preparations were applied. When water solutions were applied to the soil of potted plants or injected into the vascular system, systemic responses (involving the entire plant) occurred. Lanolin preparations containing 0.5 per cent to 2 per cent of the most effective substances caused systemic responses when they were applied to a leaf or a portion of the stem. When a leaf was dipped into a water solution of the active compounds the entire plant was affected.

Some of the most noticeable and often reported responses induced by growth substances in solution are epinasty of leaves due to increased growth on the upper side of the petiole, curvatures of stems due to unequal growth rate on opposite sides of the stem, swelling and proliferation of treated tissue, inhibition of buds, retardation of elongating parts (especially roots), the induction of adventitious roots, and the induction of abnormal geotropic responses (27, 10, 24).

A recent publication called attention to the fact that *cis* cinnamic acid and phenylacetic acid were effective when applied in the gaseous state (26). Similarities between responses induced with these two chemicals and the unsaturated carbon gases were discussed.

RESPONSES TO VAPORS OF GROWTH SUBSTANCES

It now appears that the vapors of practically all of the growth substances can be employed to induce the responses described for these same substances in solution.

The substances active when converted to the gaseous state were as follows: α-naphthaleneacetic acid, methyl α-naphthaleneacetate, ethyl α-naphthaleneacetate, acenaphthyl-(5)-acetic acid, 1-naphthaleneglycollic acid, 1-naphthaleneglyoxalic acid, α-naphthylacetonitrile, α-naphthoxyacetic acid, β-naphthoxyacetic acid, β-naphthyl mercaptoacetic acid, β-naphthyl glycine, α-naphthoylpropionic acid, β-indoleacetic acid, methyl β-indoleacetate, ethyl β-indoleacetate, indole-α-meta-β-acetic acid, β-indolepropionic acid, methyl β-indolepropionate, β-indolebutyric acid, methyl β-indolebutyrate, ethyl β-indolebutyrate, phenylacetic acid, methyl phenylacetate, ethyl phenylacetate, mandelic acid, *cis* cinnamic acid, irradiated methyl cinnamate and ethyl cinnamate, and nicotinic acid.

Epinasty, curvatures, and hyponasty. When used as described under "Materials and Methods" these substances induced curvatures and epinasty of leaves as shown in Figure 1, A and B. The most characteristic difference between the ethylene response and that of the more complex substances was bending of the stems induced by the latter chemicals. It appeared that the hydrocarbon gases might have entered the stems more uniformly and thereby did not cause unequal growth in different parts of the stem. There was some variation in the type of response according to the concentration of the vapors to which the plants were exposed. When heat was applied to the chemical to produce strong vapors, a three-minute exposure was sufficient to induce pronounced epinastic responses after the plants were removed from the chamber. The time required to induce evident responses varied with the concentration of the vapors. Thirty seconds of exposure to strong vapors of naphthalene compounds induced evident epinasty of tomato plants which remained for 24 hours. As a rule the first evidence of response to the vapors of growth substances was bending of the stem and slight downward curling of the leaves. Under the most favorable conditions this could be seen within 30 minutes. Within two hours the

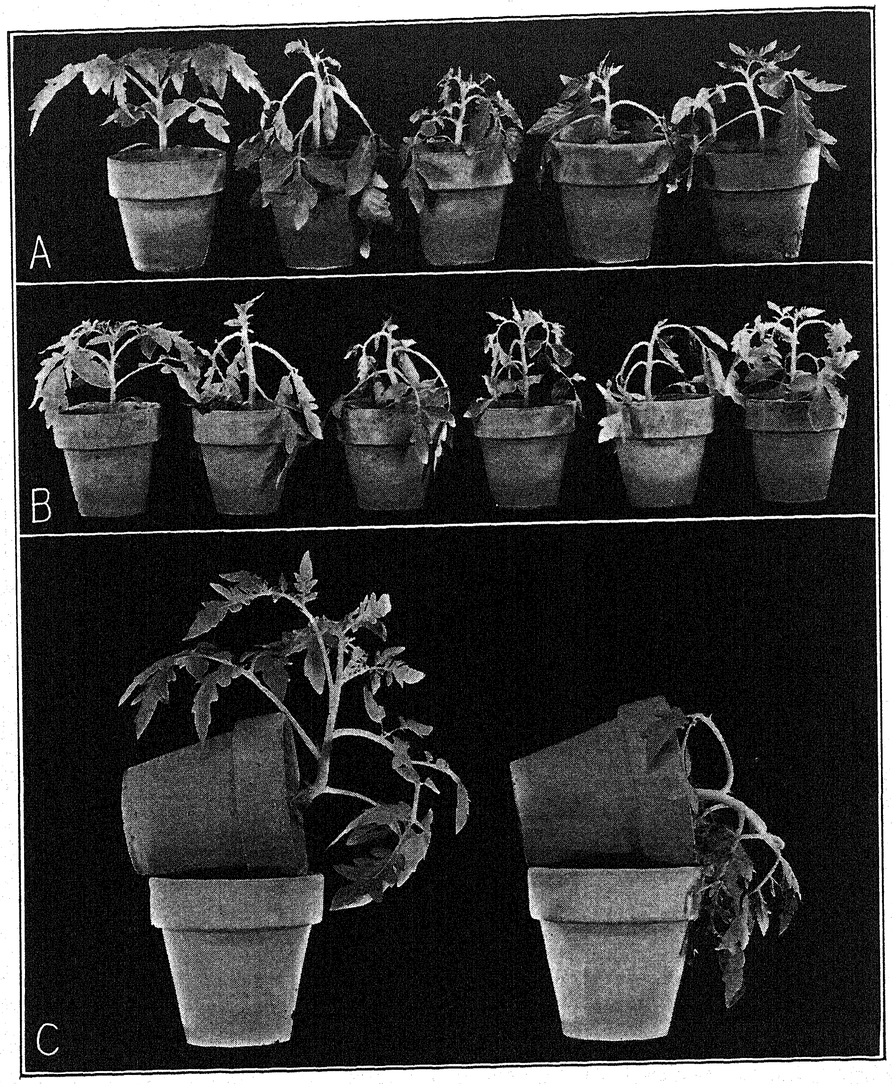

FIGURE 1. The response of tomato plants to vapors of crystalline and liquid growth substances and to ethylene gas. The plants were under bell jars during exposure. Photographs of upright plants were taken 16 to 24 hours after the exposure. The plants showing geotropic response were photographed after 72 hours. Treatments, left to right, were as follows: (A) 1. Air control. 2. Ethylene gas, 10 p.p.m. of air. 3. Naphthaleneacetic acid vapors. 4. Indolebutyric acid vapors. 5. Indoleacetic acid vapors. (B) 1. Air control. 2. Ethylene gas. 3. *Cis* cinnamic acid. 4. Ethyl indoleacetate. 5. Ethyl naphthaleneacetate. 6. Ethyl phenylacetate. (C) Positive geotropism induced with methyl phenylacetate. Control on left.

epinastic response was pronounced. Vapors of the phenyl compounds did not cause as much bending of the stem as the indole or naphthalene groups. Epinasty induced with *cis* cinnamic acid most closely resembled that of ethylene-induced epinasty. *Chenopodium* was more sensitive and made a more pronounced response to ethylene gas and to growth substance vapors than did tomato plants.

Hyponasty of the leaf blades, which is usually evident in cases of induced epinasty, was more pronounced when plants were treated with growth substance vapors than when exposed to ethylene gas.

Recovery after response. Recovery of the plants when removed from the exposure chambers varied with the chemicals involved, the concentration of the vapors, and the length of exposure. The effects were always more lasting when the naphthalene compounds were involved, and second came indoleacetic acid and its esters. Plants exposed for three minutes to a fairly high concentration of vapors of the naphthalene compounds showed only slight recovery in 48 hours. When these plants were placed in a horizontal position they could not respond to the gravity stimulus in 48 hours. In general the treated plants which recovered quickly from the epinastic response also responded to the gravity stimulus when placed in a horizontal position. The most rapid recovery occurred after exposure to low concentrations of vapors of the phenyl esters.

Tropic responses. Tomato plants were placed in a horizontal position under large bell jars and immediately exposed to vapors of growth substances. The response varied with the chemicals tested. A low dosage of ethyl indoleacetate, methyl indoleacetate, methyl naphthaleneacetate, or ethyl naphthaleneacetate caused some curling of leaves but prevented natural negative geotropism. The stem remained in a horizontal position for an hour or more and then curved downward at an angle of 25° to 45° (Fig. 1 C). This was similar to positive geotropism induced by applying a solution of naphthaleneacetic acid (10 mg./l.) to the basal end of an excised leafy shoot (25). The horizontal plants treated with a low dosage of the vapors of *cis* cinnamic acid showed negative geotropism of the stem the same as controls but the leaves on the upright part of the treated plants showed epinasty. Higher dosages caused positive geotropism, as did also the phenyl esters.

Tomato plants were inverted and suspended under bell jars with vapors of the esters of naphthaleneacetic and indoleacetic acids, the same as horizontal plants. The esters were slightly warmed and left under the bell jar for three to six hours, after which they were removed. The stems of the treated plants remained straight and the leaves showed hyponasty in contrast with controls which showed curling of leaves and negative geotropism. This response also was produced by applying the growth substances in solution to the basal end of excised, leafy shoots which were inverted (25).

Ethylene gas did not induce epinasty of inverted plants (4) as with upright plants.

Swellings and root formation. After the plants had been exposed to the vapors of growth substances they were removed to a light greenhouse so they could be observed for various growth responses. Plants which had made only slight epinastic responses recovered within a day, appearing like normal plants. Wherever pronounced epinasty occurred, recovery (leaves returning to a normal position) was slow and invariably swelling and whitening of stem tissue occurred in 48 hours and adventitious roots appeared on marigold and tomato stems and leaves in five to six days. This is similar to responses described for plants which were treated with water solutions of the growth substances.

Retardation and inhibition. Plants making a pronounced response to the indole and naphthalene compounds showed practically no recovery in the older parts for 48 hours and during this time there was very little stem elongation. As often reported for plants treated with these compounds in solution, bud growth was inhibited. Removal of the terminal bud failed to induce axillary buds to grow within a period of three weeks. Controls with terminal buds removed produced axillary shoots within one week. Naphthaleneacetic acid and its esters were especially effective for preventing growth of axillary buds. Guthrie (7) found naphthaleneacetic acid in solution and the vapor of the methyl ester inhibiting to non-dormant potato tubers.

Pea response to vapors. Etiolated pea seedlings when exposed to vapors of growth substances changed their direction of growth within six hours, showed retarded elongation, and later formed a peculiar swelling of the stem just back of the tip. This response duplicates that described by Crocker *et al.* for the action of ethylene on pea seedlings (3). The "triple response" was induced with vapors of α-naphthaleneacetic acid, methyl α-naphthaleneacetate, ethyl α-naphthaleneacetate, β-indoleacetic acid, methyl β-indoleacetate, ethyl β-indoleacetate, β-indolepropionic acid, methyl β-indolepropionate, β-indolebutyric acid, methyl β-indolebutyrate, ethyl β-indolebutyrate, phenylacetic acid, methyl phenylacetate, ethyl phenylacetate, *cis* cinnamic acid, methyl cinnamate, and ethyl cinnamate. There was some variation in the responses according to concentration, time of exposure, and the chemicals used. Figure 2 A shows abnormal geotropism which occurs within a few hours. Figure 2 B shows the same plants 47 hours after the exposure. Figure 2 C shows swelling of the stem 44 hours after exposure to five different substances. Larger swellings occur with higher dosages. Similar swellings were previously reported for sweet peas to which solutions of growth substances had been applied (27). Table I shows the percentage increase in stem diameter after etiolated pea seedlings had been treated with eight different substances in the gaseous state.

Seedlings only an inch in height remained upright after treatment but the tips became enlarged. Swellings were not limited to short stems. Several tests were made with plants 10 inches in height with similar results. Both green and etiolated seedlings showed stem swellings after exposure to the

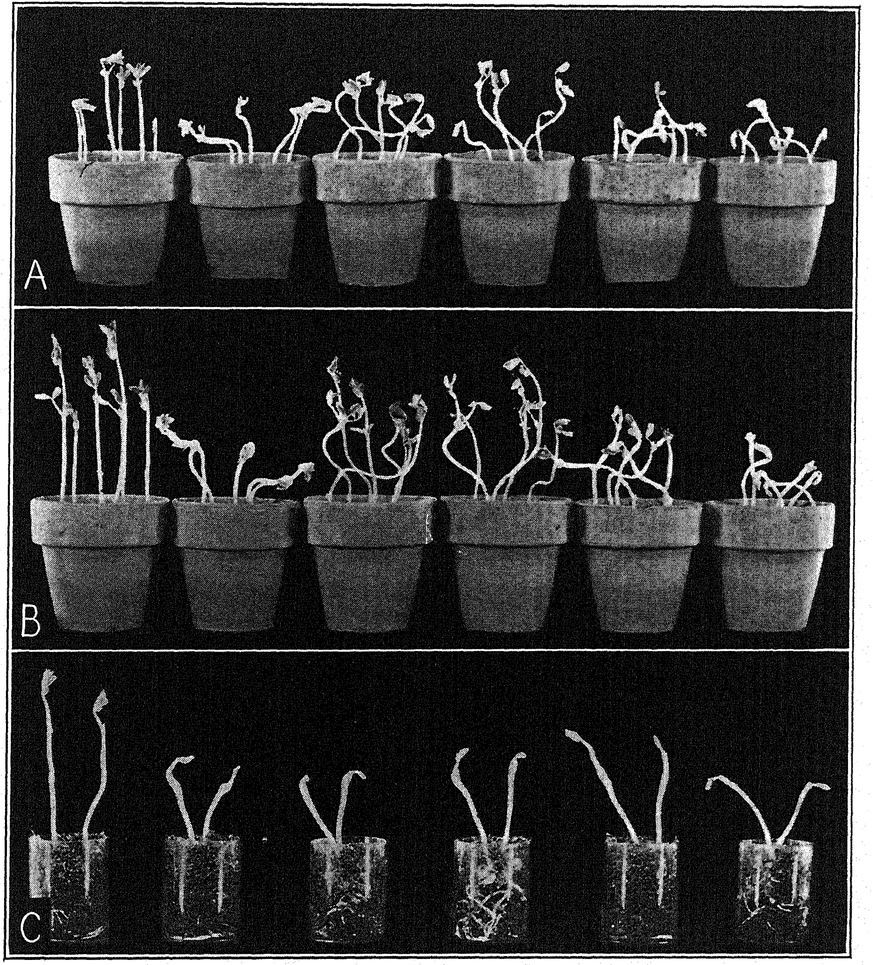

FIGURE 2. Response of garden peas to vapors of growth substances. (A) Abnormal geotropism 22 hours after exposure. Left to right: control, naphthaleneacetic acid, *cis* cinnamic acid, ethyl indolebutyrate, ethyl indoleacetate, ethyl phenylacetate. (B) Same plants as in (A) 47 hours after exposure. Note abnormal geotropism, retardation of elongation, signs of recovery, and stem swellings. (C) Etiolated peas, younger than those above, showing stem swellings 44 hours after exposure to growth substance vapors. Left to right: control, ethyl indoleacetate, naphthaleneacetic acid, methyl naphthaleneacetate, methyl indolepropionate, ethyl phenylacetate.

TABLE I

STEM SWELLINGS INDUCED BY EXPOSURE OF ETIOLATED GARDEN PEA SEEDLINGS TO VAPORS OF 8 GROWTH SUBSTANCES. MEASUREMENTS TAKEN 72 HOURS AFTER SEEDLINGS WERE EXPOSED

Chemicals	Diameters in mm. of 6 seedlings	Average	% increase over control
Control	2.0, 2.0, 2.2, 1.8, 2.0, 2.1	2.02	—
Ethylene	4.0, 4.2, 3.8, 4.1, 3.8, 3.8	3.95	96
Naphthaleneacetic acid	4.2, 4.1, 4.6, 4.7, 4.6, 4.3	4.41	118
Methyl naphthaleneacetate	4.7, 4.6, 4.9, 4.0, 4.4, 4.7	4.55	125
Ethyl indoleacetate	4.6, 4.5, 4.0, 4.2, 4.1, 4.1	4.25	110
Methyl indolepropionate	4.2, 4.0, 5.1, 5.5, 5.0, 4.5	4.71	133
Cis cinnamic	2.6, 2.7, 3.0, 3.5, 3.0, 3.2	3.00	49
Ethyl phenylacetate	4.2, 3.6, 4.7, 4.8, 4.8, 4.6	4.45	120
Ethyl indolebutyrate	3.2, 3.5, 3.7, 3.8, 3.3, 3.1	3.43	70
	Average % increase		102.6

vapors. Typical swellings occurred also on decapitated seedlings and on plants four days after the roots had been removed (14).

Corn seedlings. Etiolated corn seedlings showed abnormal geotropism when exposed to growth substance vapors. The mesocotyl appeared to be a very sensitive region, often curling and making a complete circle. Corn seedlings were especially sensitive to the naphthalene growth substances producing unusually long mesocotyls and coleoptiles. The latter outgrew the first leaf which failed to break through after the seedlings were placed in the light for three days. The rate of growth of the coleoptiles varied considerably when treated with vapors of different growth substances including ethylene. Responses induced with vapors of indoleacetic acid and its esters closely resembled those resulting from treatment with naphthaleneacetic acid though less pronounced. Etiolated corn seedlings might prove to be good test objects for detecting qualitative differences in growth substances applied as vapors.

Parthenocarpy. Potted *Ilex opaca* plants with flower buds showed parthenocarpic development of ovaries after treatment with vapors of growth substances. Flowers of control plants opened and petals curved back. Not being pollinated, fruit did not set. Opening of the treated flowers was definitely retarded. The ovary developed rapidly and the fruit set though the petals did not open. The use of vapors for ornamental and fruiting plants might be of considerable economic importance.

Mimosa response. The "sensitive" plant, *Mimosa*, was exposed to the vapors in various concentrations and time periods. The plants lost their capacity to maintain a normal equilibrium position of the leaves and leaflets. With low dosages the leaflets folded upward and with high dosages the leaf had a ruffled appearance, some leaflets being folded and others spread at the same time. When a flaming match was applied the plants appeared sluggish, making a mild response or not responding at all. In many respects the effects from growth substance vapors were similar to those described where "sensitive" plants were treated with carbon monoxide and ethylene gas (23). The gases were said to induce anaesthesia. Both vapors of growth substances and gases induced systemic responses in contrast to local effects where a single set of pulvinal cells was treated with a lanolin preparation of a growth substance (27).

The vapors of naphthaleneacetic acid and its esters were the most effective for "desensitizing" *Mimosa* plants. A three-minute exposure to a high concentration caused a pronounced effect which lasted for several days.

DISCUSSION

The results of these experiments show that vapors of solid and liquid growth substances induce the same types of responses as those resulting from the application of the same chemical compounds directly to the tissue as water solutions or mixed with other suitable carriers. It thus appears that regardless of the physical state of the compounds, they induce the same types of physiological activity when applied under suitable conditions to sensitive test plants. The similarity between certain unsaturated carbon gases and the principal crystalline growth substances has been previously pointed out (2). It was shown, also, that such gases as ethylene were effective when dissolved in water or in lanolin (27).

The shortest time exposure given to plants was 30 seconds. In that test with vapors of naphthaleneacetic acid tomato plants showed practically as much epinasty as that induced with 30 minutes' exposure. To volatilize naphthaleneacetic acid a watch glass holding the crystals was set on a hot, inverted crucible. The vapors in the air surrounding the plants decreased as the crucible cooled. The more volatile substances, requiring only one drop of the chemical without heat, acted more like the unsaturated gases. A small amount of the esters of phenylacetic acid and *cis* cinnamic acid were applied for several hours, inducing pronounced responses without injury.

To activate commercial methyl and ethyl cinnamate with ultra-violet light the esters were placed in a Pyrex Petri dish and irradiated through the cover. The first few tests with the irradiated esters gave negative results. Then the lid of the Petri dish was placed under a bell jar with tomato plants and a pronounced epinastic response occurred. It appears, therefore, that the *cis* form of the esters is very volatile, arising from the liquid and settling on the cover of the dish while the chemical is being irradiated.

There has been considerable opposition (21, p. 181, 182, 191) to the suggestion that ethylene might be a natural hormone, notwithstanding the fact that fruits and growing plants produce ethylene in comparatively large amounts (5, 16, 19). In fact, ethylene is the only natural plant hormone-like substance that has been definitely identified with accepted chemical methods (1, 6, 15, 17, 18). This statement is made despite the often mentioned "auxins a and b" which have not been synthesized or even chemically identified from green tissue. The practice of formulating theories based on the assumption that "auxin a" is "the plant hormone" (20) has been previously discussed (10) and further criticized (11, 12, 22) in reviews of the book by Went and Thimann (21). From the available information it appears that the existence of "auxin a" in plants is only hypothetical so far as actual chemical compounds are concerned. Therefore, the suggestion that ethylene be given consideration as a possible natural hormone-like substance may not be out of place.

One of the best known reactions to ethylene gas is the so-called "triple response" of leguminous seedlings described by Crocker, Knight, and Rose (3). This response consists in reduced rate of elongation, abnormal geotropic response, and increase in diameter of the stem. This same triple response was induced with vapors of growth substances. Both etiolated and green garden pea seedlings made the "triple response." The degree of response varied with the dosage of the vapors the same as with ethylene gas. There was some difference in the rate of response with the different chemicals. The esters of naphthaleneacetic acid appeared to be the most active for causing retardation of stem elongation and swelling of the stem.

In a recent publication Michener of California Institute of Technology sought to define the action of ethylene on plants. He states (14, p. 719), "The experiments with oat and pea seedlings indicate that ethylene causes an activation or accumulation of food factor or some other factor promoting growth."

We agree especially with the latter part of this statement since it shows we are far from an understanding of how ethylene acts. Again in attempting to explain why ethylene causes stem swellings Michener says, "First as was previously indicated auxin in low concentrations is a prerequisite for the formation of swellings; for ethylene does not induce swelling formation when the supply of auxin is completely removed."

The same statement could safely be made for any other factor as yet unknown or for known factors essential for growth as, for example, sugar, many carbohydrates, nitrogen, etc.

In discussing the necessity for roots on seedlings, Michener states, "Possibly this preparatory reaction involves the production of a substance or substances in the roots which are necessary for the formation of swellings, but it may also be some other type of reaction."

We agree with the latter part of the quoted sentence. When so many different substances, of simple or complex structure, induce indistinguishable responses in plants it is sure to be difficult or perhaps impossible to determine how the mechanism for one response differs from that of another.

To dispose of the problem of explaining downward bending of *Vicia faba* stems when treated with ethylene, Went and Thimann (21, p. 160) cite the work of van der Laan who claimed that the response is correlated with an unequal "auxin" distribution such that the upper side has more than the lower. With this assurance Went and Thimann say, "The curious behavior of these seedlings in ethylene is thus explained." Went and Thimann (21, p. 182) give the same explanation for epinasty of leaves. They say, "It is most probable, therefore, that the effect of ethylene is to influence the distribution of auxin in the petiole."

Now that vapors of "accepted" growth substances (indole, naphthalene, and phenyl compounds) induce the same abnormal, geotropic response as ethylene gas, the explanation given above is not acceptable. Furthermore, Michener (14) has not been able to verify the work of van der Laan (13).

In conducting experiments with vapors of growth substances it was necessary to wash thoroughly the bell jars before starting new experiments. For example, after a plant was exposed under a bell jar to vapors of ethyl naphthaleneacetate, enough of the active substance clung to the wall to induce a response when new plants were inserted. Similar responses were induced with new plants for three days without the introduction of more of the substance. Similar responses occurred under bell jars where the esters of phenylacetic acid, cinnamic acid, and the indoles had been used.

When the esters of phenylacetic acid were placed on a warm iron under bell jars with plants, enough vapors escaped to cause injury. If the dosage was too high no epinastic response occurred, but leaves were damaged.

In one experiment the ethyl naphthaleneacetate was heated enough to cause fumes to arise and flow over the plants. At this point the container holding the substance was removed. The plants made a pronounced response showing they had absorbed the substance and that the compound had not been destroyed by heat. The plants were removed to the greenhouse and observed for several days. Leaves and stems became greatly swollen within 48 hours and finally many roots developed on both stems and leaves. Stem elongation was practically stopped for ten days and the growth of axillary buds was inhibited. Within two weeks the terminal bud slowly resumed its growth. It was evident, therefore, that the degree of response varied with the dosage of the substance.

There was one striking difference between the esters of phenylacetic acid and those of the indole and naphthalene substances. The phenyl compounds in high dosages damaged the leaf blades. The other growth substances did not so affect the leaves. Similar results have been noted where high concentrations of these chemicals were used in water or lanolin.

For the most part, the responses dealt with in this paper have concerned concentrations of the substances below the lethal limits. A positive test for all the active substances consisted of responses induced with sublethal doses.

Another difference was noted in connection with recovery. Plants showing induced epinasty from the phenyl compounds recovered more quickly than those treated with the indole or naphthalene substances.

Epinasty induced with nicotinic acid differed from that of all the other substances. There was more curling in the youngest leaves and the older ones were slow to develop epinastic response. The response was evident in 24 hours and pronounced in 48 hours.

Not enough work has been done with horizontal and inverted plants exposed to vapors of growth substances to determine how closely the responses resemble those induced with the same chemicals in solution. The few experiments conducted show, however, that horizontal stems can be made to curve downward, grow straight, or turn away from the earth according to the concentration of the substances.

The *Mimosa* plants have not been as completely "desensitized" with the vapors of growth substances as with ethylene or carbon monoxide gas. Heavy vapors of the indole and naphthalene compounds were the most effective. The gases cause a much more ruffled appearance of the leaves than the vapors. There was a lack of correlation when the plants were treated with vapors but the appearance was not as striking as that of gas-treated plants. In all cases the pulvinal cells at the base of the petiole lost their capacity to respond to stimuli more readily than those of the leaflets.

One chemical, naphthalene propionic acid, which shows slight physiological activity when applied in solution, did not induce a response when applied as vapors under the conditions of our experiments. In general, however, it may be said that acids and esters of physiologically active substances may be applied in solution or in the gaseous state.

For the experiments reported only plants showing a response to ethylene gas were used. Of the marigold, cosmos, tomato, and *Chenopodium*, the latter is the most sensitive to low dosages. The marigold is more sensitive than the tomato but due to the ease of growing and handling, the tomato was selected for most of the experiments. Soon it is hoped that plants not sensitive to ethylene gas can also be tested with vapors of growth substances. Also, very likely many other compounds will prove effective when applied as vapors. Several compounds included in this report have not been previously considered as growth substances.

SUMMARY

There is presented a review of the responses of plants to growth substances applied in solution and to unsaturated carbon gases.

Twenty-nine compounds which were physiologically active as plant growth substances when applied in solution were found to be active also when applied as vapors to the following plants: tomato, corn, Klondike cosmos, marigold, girasole, *Mimosa*, *Chenopodium*, and garden pea. Plants exposed under bell jars or large beakers to vapors of the substances showed practically all the responses previously reported for these compounds applied as solutions. Characteristic responses were induced with exposures as short as 30 seconds.

The well-known "triple response" of etiolated pea seedlings commonly associated with the effect of ethylene gas was induced with vapors of 17 growth substances.

Excessive elongation of etiolated coleoptiles and mesocotyls of corn occurred when the seedlings were exposed to vapors of these substances in light or in darkness. Pronounced curling and swelling of the nodes were also noted.

The similarities between responses induced with the unsaturated hydrocarbon gases and vapors of growth substances are discussed.

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A NEW FRAGRANT GLADIOLUS HYBRID

FORMAN T. MCLEAN

Four generations of breeding gladiolus for fragrance resulted in the large-flowered hybrid exhibited by the Boyce Thompson Institute for Plant Research, Inc. at the 1939 International Flower Show at New York, N. Y. It took three generations to build up a small fragrant winter-growing hybrid capable of transmitting strong fragrance to its offspring, and in this process the Mendelian inheritance of four factors for fragrance in this small-flowered race was worked out (1, 2).

The strongly fragrant small gladiolus was crossed with a commercial variety of garden gladiolus, which gave to the resulting hybrid size, vigor, color, and form. The new fragrant hybrid thus combines the fragrance derived from the small winter-blooming species hybrid "Sweet glads," with the general appearance of the garden gladiolus. It also has the winter-blooming habit, flowering at a cool temperature and with short days, without extra light in the greenhouse. Whether it will prove an equally good summer bloomer in the garden remains to be tested.

The pedigree of this new hybrid, now designated by its record number —Z-36—is simple, being derived on the one hand from a garden hybrid gladiolus "Gretchen Zang," as pollen parent, and from two clones of wild species, *Gladiolus tristis* L. concolor I and *G. recurvus* L. bronze as great grandparents on the other.

First, these two species clones were crossed, producing a series of hybrids designated serially from 60–1 to 60–221 inclusive. These were almost all mildly "violet" fragrant, like the *G. recurvus* parent. Then one of these, 60–17, was back-crossed on the *G. tristis* concolor parent, producing a "lemon" scented hybrid, 152–15, which otherwise appears exactly like the night scented *G. tristis* concolor I. Two others of the 60- series were intercrossed to produce a cream-colored "violet" scented second generation hybrid, 157–1. By crossing 152–15 with 157–1 a series of strongly scented hybrids resulted, the most fragrant of which was 198–11, a small streaky lavender flower with short stature and few flowers. The fragrance is of a new type, designated "pungent," and much resembling that of a carnation.

The garden gladiolus "Gretchen Zang" was then crossed with 198–11, resulting in more than 37 seedlings, two of which have flowered. The first to flower, Z-36, bloomed last year in early March, 1938, and was the first fragrant hybrid of the large-flowered gladiolus type to bloom (Fig. 1).

Previous summer-blooming hybrids such as 113–11, a mildly "violet"

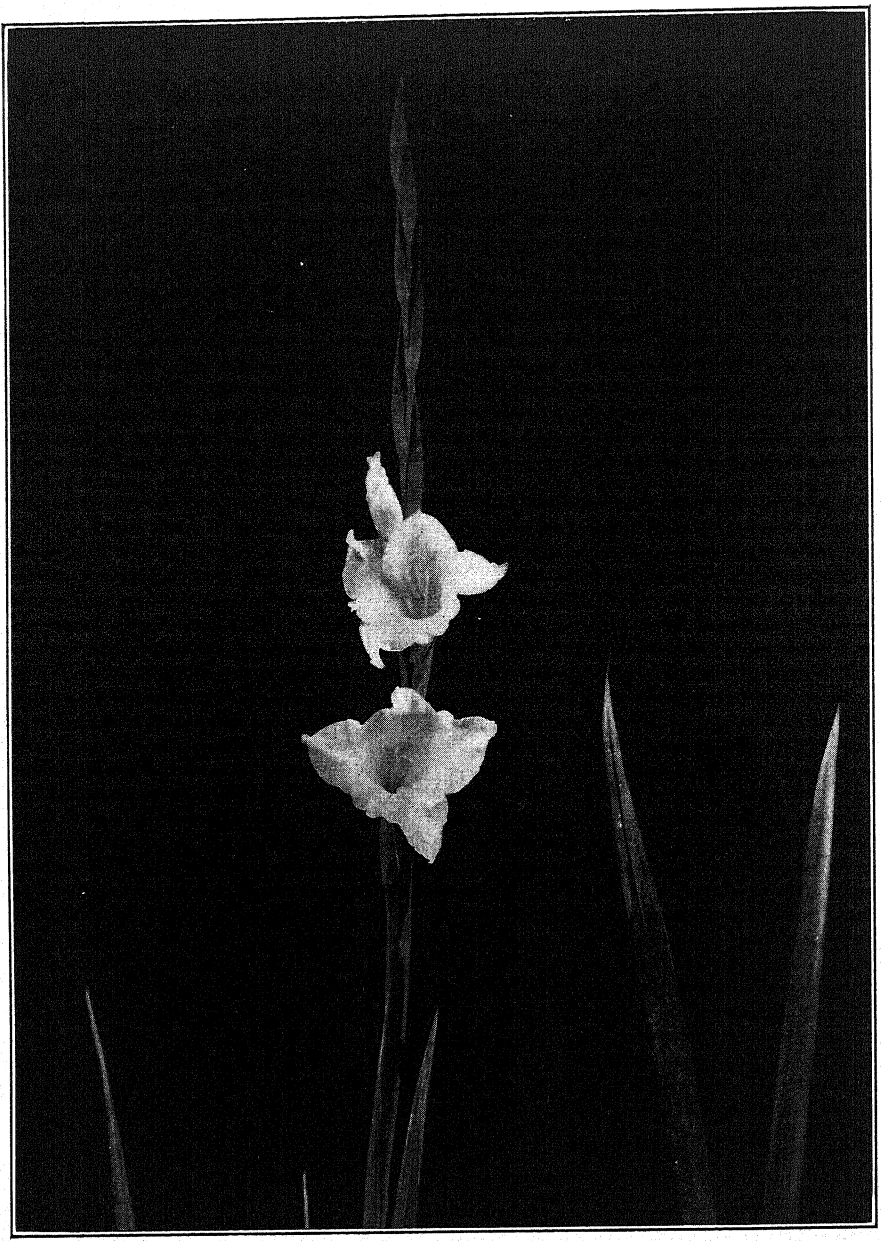

FIGURE 1. A new fragrant gladiolus, Z-36. (1/3 natural size.)

scented, small, mustard-colored flower, which won the first award for the most fragrant gladiolus in 1936 at the Metropolitan Gladiolus Society Flower Show at New York, N. Y. in competition with "Incense" and other faintly fragrant hybrids, and 240–1, since named "Rose Red," which won a similar award in 1938, are both dwarf plants and small-flowered, with few flowers on a spike, and not to be compared with the ordinary garden gladiolus.

Z-36, on the other hand, is to all outward appearance a typical small decorative type gladiolus with well formed, ruffled, open three-inch flowers, borne 10 to 13 on a 33-inch stalk. The flowers are well spaced and faced, with about four open at once. The color is creamy white, tinted rose on the edges, and with a distinct yellow blotch in the throat. The fragrance is classed as "violet" and like all matters involving taste or smell, occasioned some difference of opinion at the Flower Show.

On March 16, 1939, John M. Arthur took a record of people who smelled the flowers of Z-36, as to whether they found it strongly fragrant, slightly so, or non-fragrant. This the author continued on March 17 and 18, adding two other flowers of gladiolus for comparison.

In all, 702 people smelled the flower of Z-36 and recorded their findings, after it was carefully explained to them that some found it fragrant and some did not. Of the total number who reported, 547 found it strongly fragrant, 20 found it weakly so or were doubtful about it, and 135 found it to be non-fragrant. Thus, 77.9 per cent of the people found it distinctly fragrant, 20.6 per cent found it scentless, the remaining 1.5 per cent being doubtful.

To further test the ability to smell these flowers, two other gladioli were introduced into the test on March 17 and 18: a scentless flower of *G. tristis* (which is normally strongly night scented) and a "lemon" scented hybrid, 381–5. All of the observers in these two days agreed that the *G. tristis* was scentless and several were surprised, recognizing the flower as of a species they knew to be normally sweet. Of 476 who reported on 381–5, 352, or 74 per cent, found it fragrant, and 88, or 18.5 per cent, found it not fragrant.

Thus, in the case of each flower, nearly three-quarters of the people found it fragrant. Of those who did not, practically all of them found one or the other flower fragrant. So it was not lack of acuteness of the sense of smell, rather a specific idiosyncrasy for the particular kind of odor which caused the difference.

It now appears that because of the difference in sense of smell, a flower must have more than one type of fragrance in order to appeal to everybody as fragrant.

Breeding gladioli for fragrance has resulted in a fragrant gladiolus hybrid of good form and character, and there is a reasonable expectation that others, having all of the range of color and form found in the garden hybrids, will result from similar hybridizing.

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DEVELOPMENT OF DORMANCY IN LILY BULBS[1]

NORWOOD C. THORNTON

For many decades greenhouse forcers of Easter lilies have experienced considerable trouble with bulbs that upon being planted produce either no growth or very poor, undesirable, and undersized plants which flower from one to ten months later than normal plants. In some cases bulbs have been observed to remain dormant for as long as one year after planting. Usually these bulbs will produce desirable root systems yet the stems will not elongate. Since this trouble occurs to the greatest extent with bulbs from Japan which are usually shipped packed in finely pulverized subsoil in tight wooden cases, it seemed possible that the bulbs were not obtaining sufficient aeration and that this might be an important factor in the production of the abnormal plants from many of these bulbs. The behavior of these plants suggests a condition similar to that already reported (2) in that normally non-dormant embryos of seeds of the cocklebur can be made dormant by storage under conditions limiting or entirely eliminating the oxygen supply to the seeds. When placed under favorable conditions for germinating after storage the seeds were very slow to germinate and the plants produced were dwarfed with abnormally deep green foliage. After a growing period of many weeks the plants from seeds that had been stored in a minimum of oxygen were only a fraction of the height of plants from seeds that had been stored in a similar manner but exposed to a normal supply of oxygen.

Because of the importance of this problem from the standpoint of both the commercial grower or handler of bulbs and the scientist investigating lily growth problems, some experiments were set up with a limited quantity of bulbs grown at the Institute[2] to test the effect of various storage conditions upon the production of plants from these bulbs following storage.

MATERIAL AND METHODS

Bulbs of *Lilium longiflorum* Thunb. variety Erabu planted May 17, 1938, were harvested on four different dates in 1938: August 17 (few in flower though most flowers had been dropped, bulbs small), September 6 (all blooms gone, seed pods were filling), October 7 (practically mature, seed was not quite ripe for harvest, but pods were beginning to soften), and November 1 (seed pods dry, plants dying). These bulbs were allowed

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[2] Thanks are extended to E. P. Imle, Fellow of the Lily Disease Investigation supported jointly by the Boyce Thompson Institute for Plant Research, Inc. and Cornell University, for furnishing the bulbs used in this investigation.

TABLE I

CONDITION OF LILY BULBS WHEN REMOVED FROM STORAGE

Month harvested, 1938	Days in storage	Treatment of each lot of 12 bulbs during storage			
		Stored in soil		Stored without soil	
		Container not sealed	Container sealed	5% O_2	40% O_2
August	95	Dried slightly; no growth	Good condition; no growth	6 bulbs rotten; 6 bulbs rotting at base	All 10 bulbs sprouting with roots; scales sprouting
September	84	Good condition; some rooting	Good condition; no growth	Good condition; no growth	7 bulbs sprouting; all bulbs with roots
October	68	Good condition; 6 bulbs sprouting and rooting	Injured	Good condition; no growth	12 bulbs sprouting and with roots
November	41	Good condition; some rooting	Good condition; no growth	Good condition; no growth	4 bulbs starting to sprout; some root growth

TABLE II

TIME REQUIRED TO PRODUCE NEW GROWTH ABOVE GROUND AFTER BEING STORED UNDER VARIOUS CONDITIONS AT 75° TO 78° F.*

Month harvested, 1938	Treatment given during storage							
	Stored in soil				Stored without soil			
	Container not sealed		Container sealed		5% O_2		40% O_2	
	Days for emergence of		Days for emergence of		Days for emergence of		Days of emergence of	
	First sprout	Sprouts from all	First sprout	Sprouts from all	First sprout	Sprouts from all	First sprout	Sprouts from all
August	26	36	26	**	—	—	†	18
September	25	36	34	52	25	32	6†	11
October	18	34	—	—	20	23	2†	20
November	13	26	32	55	20	29	11†	32

* Average of duplicate plantings.

** Five bulbs in one box and two in another sprouted in 32 days, the remaining bulbs stayed dormant for 153 days.

† Considerable growth during storage before planting.

to dry at 75° to 78° F. for seven to nine days before they were stored. The bulbs harvested in August were stored 94 days and planted in soil November 28, bulbs harvested in September were stored 84 days and planted December 6, and bulbs harvested in October and November were stored 68 and 41 days respectively and planted in soil December 22. Twelve bulbs were used in each test and at the end of the storage period duplicate lots of six bulbs each were planted in boxes of fertile soil and grown (without further fertilization) in the greenhouse at a minimum night temperature of 65° F.

The bulbs from each harvest were divided into four lots and placed in four-gallon tin containers in preparation for storage. The first lot was held as a control where the bulbs were thoroughly covered with the pulverized subsoil which had been used to pack bulbs shipped from Japan, but the container was not sealed. The second lot was packed the same as the first lot except that the can was sealed with a tin cover and the seams were filled with melted paraffin. The third and fourth lots were placed in similar containers, without the soil but sealed with covers having inlet and outlet tubes in order that the storage atmosphere might be renewed frequently. The first and second lots, open and sealed in soil, were placed in storage at 75° to 78° F. and were not disturbed until the end of the period. The containers of the other two lots were removed from this storage every seven days in order to renew the original oxygen content of the storage atmosphere, 5 and 40 per cent in lots three and four respectively. Although this amount of oxygen was not constantly maintained, these bulbs were exposed to a low and a high per cent of oxygen at regular intervals in order that they would not be exposed to constant anaerobic conditions such as would eventually develop in a sealed container in which the gas was not changed during the period of storage.

No period of storage at low temperature was employed since it was the object of these tests to determine the effect of insufficient aeration during storage for various periods of time at a moderately high temperature which would duplicate in part some of the conditions under which the bulbs are shipped.

RESULTS

When removed from the various storage conditions in preparation for planting, the bulbs were found in most cases to have kept very well as indicated by the information presented in Table I. Three of the four lots of bulbs held in soil in the open container were commencing to grow while those held in soil in the sealed container had not produced any growth. A large percentage of the bulbs held in 40 per cent of oxygen had produced both top and root growth while the bulbs held in 5 per cent of oxygen had not produced any growth. Two lots of bulbs (in soil in sealed container and in 5 per cent O_2) were found to be injured and, although they were planted, no growth was obtained because the bulbs rotted in the soil.

As shown in Table II the emergence of sprouts from bulbs stored in soil in the open container took place at fairly uniform rate for the early harvested bulbs. And as the bulbs became more mature, i.e. harvested in October and November, the rate of emergence of new growth was somewhat faster than that obtained from bulbs harvested in August and September. It is apparent that the more mature bulbs receiving a shorter period of storage produced an earlier renewal of growth after storage. These results with bulbs stored in the usual manner are quite significant when it is considered that usually the longer Easter lily bulbs are held in storage the quicker they come up after planting and the earlier they bloom.

In contrast to the open soil storage the bulbs stored in soil in closed containers were, in general, slower to produce new growth. Although the August harvested bulbs started to grow in 26 days, only five bulbs in one box and two bulbs in another box produced growth during the period of the test. When the remaining five bulbs were dug up 153 days after planting they were found to be in perfect condition but without any evidence of top growth and very little root growth. In addition to the fact that plants were not obtained from some of the bulbs, those plants which were produced were abnormal in that the stalks were short and the leaves were very dark green in color as is characteristic of plants produced from partially dormant bulbs or seeds. The September and November harvested bulbs (October bulbs were injured in storage) required 32 to 34 days to produce the first sprout and 52 to 55 days for complete sprouting to take place. Although all of these bulbs produced plants, the plants were characteristically short and very dark green in color.

Bulbs that received 5 per cent of oxygen produced the first plant above ground in 20 days and complete sprouting in a maximum period of 32 days. In comparing the growth from bulbs harvested at the same time, the 5 per cent of oxygen treatment was not quite as good as open-soil storage but far better than sealed-soil storage.

Storage of the bulbs in 40 per cent of oxygen appeared detrimental because of the short growth produced while still in storage. However, when these bulbs were transferred to soil, growth continued at a rapid rate with August, September, and October bulbs, which produced complete sprouting in 18, 11, and 20 days respectively. The November harvested bulbs were slightly slower in growing, requiring 32 days for complete sprouting, due no doubt to the fact that these bulbs had a very short period of rest after harvest. Here also all the plants produced were normal in every respect and, in fact, these were the tallest plants that were produced in these tests.

On March 30, photographs, which are shown in Figure 1, were taken of

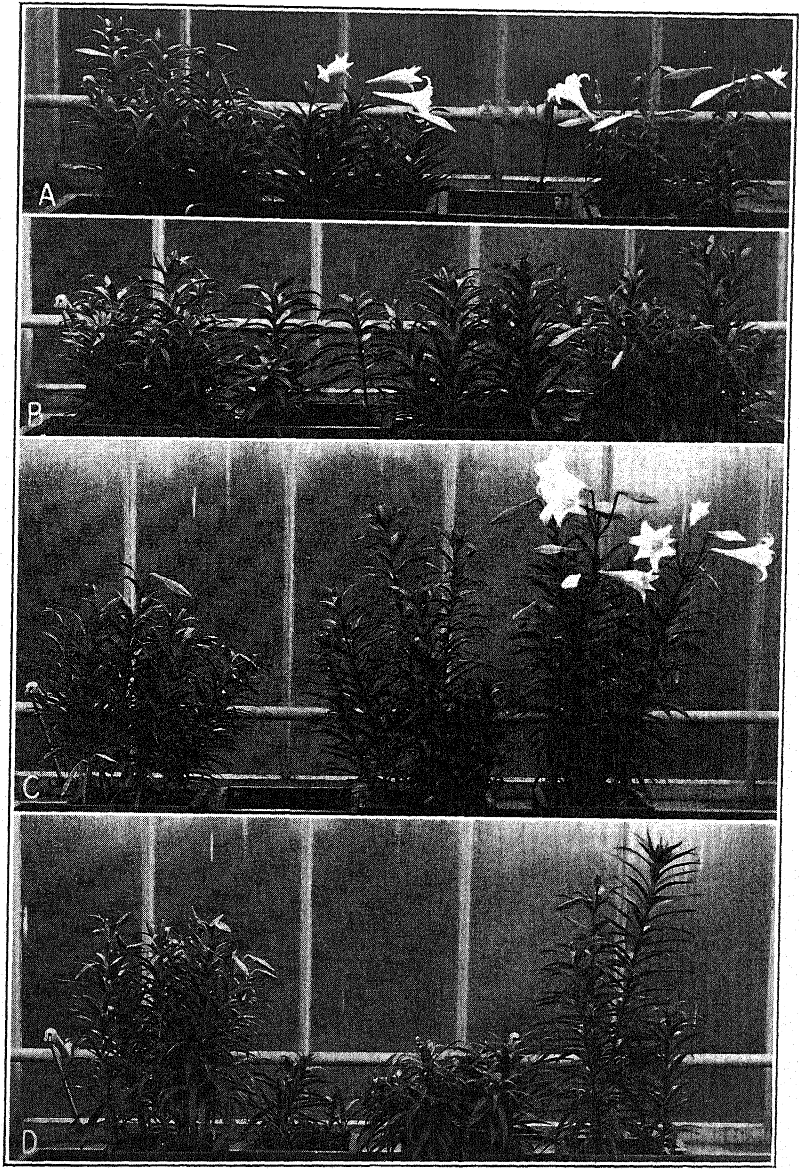

FIGURE 1. Plants produced by March 30 from bulbs stored (left to right) in soil in open container; in soil in sealed container; without soil in sealed container with 5 per cent oxygen; and without soil in sealed container with 40 per cent oxygen. (A) Bulbs harvested August 17, stored, then planted again November 28; (B) bulbs harvested September 6, stored, then planted December 6; (C) bulbs harvested October 7, stored, then planted December 22; (D) bulbs harvested November 1, stored, then planted December 22.

all the boxes of lilies and from these pictures one can estimate the relative rate of growth of plants harvested at different periods and stored in various ways. Bulbs harvested in August (Fig. 1 A) and stored in soil in an open container required 122 to 135 days to grow to an average height of 16 inches and produce an average of 2.5 flowers per stalk. And the bulbs stored in soil in sealed containers required 126 to 135 days to grow (five bulbs in one box and two in another box and five remaining bulbs dormant as previously discussed) to an average height of 11 inches and to produce an average of 1.8 flowers per stalk. As more mature bulbs were used, i.e. harvested in November (Fig. 1 D) only 82 to 116 days were required by bulbs stored in soil in open container to produce an average height of 20 inches and an average of 2 flowers per stalk. In contrast, the bulbs of the same age but stored in soil in a sealed container required 144 days to produce an average height of 12 inches and an average of 0.6 flower per stalk. Thus we see from the data and from Figure 1 that more mature bulbs produce the best growth following storage and that sealed storage, where aeration is at a minimum, is detrimental not only to the production of good growth of plants but also to the production of flowers by these plants.

In general, bulbs stored in 5 per cent of oxygen required a period of growth after sprouting intermediate between that required for bulbs stored in soil in the open and closed containers. For a short period of time during the early stage of growth the October and November harvested bulbs that were stored in 5 per cent of oxygen had some characteristics of partially dormant plants similar to those produced by bulbs from the sealed storage lots. This is noticeable with the plants in the third box from the left in Figure 1 C and especially so in Figure 1 D. However, these plants soon overcame this retarded growth and as shown by most of the plants in Figure 1 C they produced approximately the same average total growth and number of flowers per plant as obtained from bulbs of the same maturity but stored in soil in an open container. These results indicate that the lily bulbs will withstand considerable reduction in the concentration of oxygen during storage before the condition of dormancy develops to such an extent as to be seriously detrimental to the production of good plants.

With bulbs stored in 40 per cent of oxygen the period of growth preceding flowering, the average height of the plants, and the average number of flowers per plant were approximately the same as produced by bulbs of the corresponding maturity that were stored in soil in the open container.

DISCUSSION

These results indicate that if immature (early harvested) bulbs are subjected to poor aeration during storage there is a tendency for some of the bulbs to become dormant and not produce growth when planted in the soil. Although some of these bulbs will produce growth at nearly the same rate as those stored under conditions that provide some aeration the plants will be somewhat shorter than plants from bulbs that were stored under the latter condition. If, however, mature (late harvested) bulbs are subjected to poor aeration during storage, the renewal of growth when planted is much slower than when immature bulbs are used and likewise a longer period of growth is needed to produce a plant with flowers than if the bulbs had been stored under conditions providing for aeration.

Although this investigation has dealt with aeration from the standpoint of oxygen supply it is possible that the accumulation of carbon dioxide from respiration may have been effective in bringing about some of the results obtained so that this point must be investigated further. In the case of cocklebur seeds (2) it was observed that long periods of storage in carbon dioxide in the absence of oxygen caused physiological breakdown of the seeds that was akin to decay. And it is possible that a similar condition existed in the present investigation when some of the bulbs were lost due to rotting in storage. In this connection, Green (1) observed that lily bulbs packed in finely divided soil decayed during transit from Japan to England while those packed in coarse soil arrived in good condition. By storage of the bulbs in an atmosphere of carbon dioxide he was able to produce a decay similar to that observed in bulbs packed in pulverized soil and from this he concluded that lily bulbs died because the oxygen of the storage atmosphere was largely replaced by carbon dioxide. Green, however, did not report any difficulty in the growth of plants from bulbs stored in either the fine or coarse soil.

Since lily growers have experienced considerable trouble growing plants from bulbs packed in the finely divided subsoil and practically no trouble with bulbs packed in peat or other coarse packing media, the practical evidence correlates very well with these experimental results which show aeration to be an important factor in bulb storage. In cooperation with E. P. Imle, further investigations of various storage conditions for lily bulbs are to be continued.

SUMMARY

1. *Lilium longiflorum* Thunb. bulbs stored in soil under conditions limiting aeration develop partial dormancy and when planted in soil these bulbs produce either no growth or very slow-growing undersized plants with abnormally deep green foliage characteristic of dwarfed plants.

2. Storage of the bulbs in soil under conditions providing for aeration or in containers supplied with oxygen eliminated the problem of dormancy since the plants grew rapidly to a good height and produced a satisfactory number of flowers.

3. Bulbs harvested late in the season and stored in soil in an open container for a short period of time renewed growth and produced flowers sooner than bulbs harvested earlier in the season and stored in the same manner for a longer period of time.

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UNUSUAL PHYSIOLOGICAL RESPONSES INDUCED ON INTACT PLANTS BY CAPPING WITH BLACK CLOTH

A. E. Hitchcock and P. W. Zimmerman

INTRODUCTION

It was recently shown that the force of gravity stimulated the plant when oriented for a time in an abnormal position, causing epinasty of leaves and proliferations on stems and leaves (9, p. 316, 324). In more recent experiments it was found that subjecting a part of the tomato plant first to darkness for six days or longer and then to light, induced epinasty of leaves, pronounced swelling and proliferation of stems and petioles, the formation of adventitious roots, and disturbed considerably apical dominance and other correlations. Thus the responses induced are similar to those caused by the application of synthetic growth substances. The magnitude of the responses indicates that they were caused by an increase in the production of natural hormones and not as a result of a redistribution of the existing hormones at the time of capping.

The present paper reports data which constitute additional evidence in support of the view that applied growth substances such as indoleacetic acid and other accepted growth substances are *not specific* for the responses they initiate, since the stimulus of gravity or of light may be substituted for the stimulus of an active substance. Much confusion in the literature has resulted from the failure to distinguish between substances which are active for initiating a reaction and those which constitute an important link in the mechanism of the reactions which follow.

METHODS

Tomato plants five to ten inches in height were partially covered with black cloth caps for periods of 3 to 14 days. Generally the upper one-third to one-half of the plant was covered as shown in Figure 1 A, center. In certain tests the stem was wrapped with a one-inch strip of black cloth for a distance of about two inches below the growing tip but in such a manner as to allow the tip and upper leaves to continue growth in light (Fig. 1 A, right). Thus in both types of dark treatment the principal region of elongation was covered. Unless otherwise stated the plants were grown on the greenhouse bench during the dark period and were transferred to a Wardian glass case after the caps were removed. Two methods were used for supporting the black cloth caps. In one case rigid stakes were inserted in the soil in a vertical position next to the stem and with the upper end slightly above the top of the plant. A second method consisted of supporting the caps from above by means of wires.

Bonny Best and Marglobe varieties of the tomato (*Lycopersicon esculentum* Mill.) were used in these tests.

RESULTS

Epinasty. Within 24 hours actively growing Marglobe tomato plants exhibited epinasty of one or more leaves located below the region capped

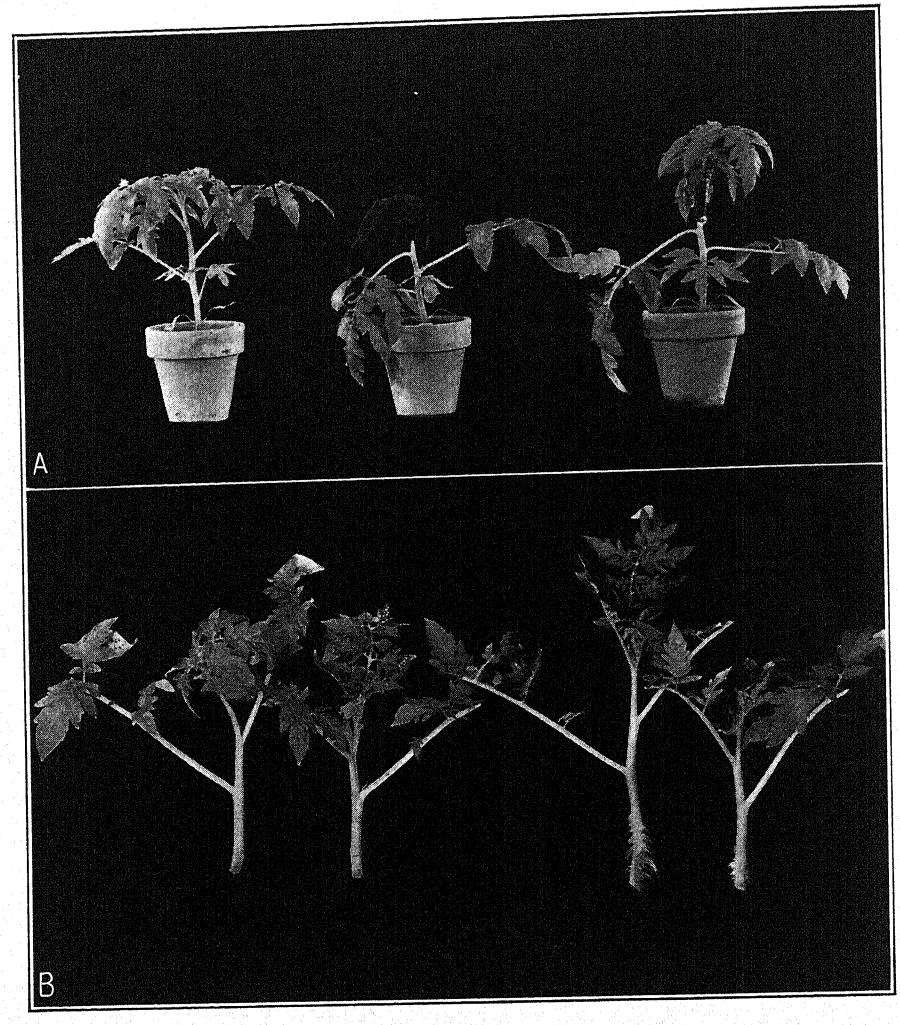

FIGURE 1. Physiological responses induced by covering the upper parts of tomato plants with black cloth. (A) Control (left). Epinasty of leaves after 48 hours on capped plant (middle) and on plant wrapped with black cloth (right). (B) Left, cuttings made from control plants. Right, cuttings made from plants capped for 7 days then planted in the rooting medium for 4 days.

or wrapped with black cloth (Fig. 1 A, center). In the case of plants wrapped as illustrated in Figure 1 A, right, one or more of the leaves above the wrapping exhibited noticeable downward curling. Such leaves were more rigid than similar ones on control plants, regardless of the degree of curvature. These epinastic responses were typical of those induced by the unsaturated carbon gases and by other active growth substances (e.g. indole, naphthalene, and phenyl compounds) applied as solids, liquids, or vapors. The recovery of leaves showing an epinastic response was similar to that reported for plants exposed to low concentrations of ethylene (2), that is, all leaves did not fully recover, but some of the leaves showed nearly complete recovery.

Swelling and proliferation. Plants capped with black cloth for three days or longer resembled in appearance tomato plants treated with growth substances (11). The dark treatment caused loss of the normal green color and various types and degrees of swelling and proliferation which varied with the duration of the capping, the relative age and condition of the plant, and the length of the region capped. Generally, the most pronounced responses occurred at or near the base of the capped region (Figs. 1 and 2). In some cases the entire portion of stem under the cap had become blanched and swollen.

Microscopical examination of cross sections made through the proliferated region, illustrated in Figure 2 C, revealed the presence of disorganized masses of abnormally large thin-walled cells. No attempt was made to determine their exact origin, and cross sections were not taken of similar tissue at an earlier date.

The etiolated upper parts of capped plants regained their green color very slowly when exposed to light either on the greenhouse bench or when transferred to higher humidities in Wardian cases. Blanched regions showed little change in color for several to many days, depending upon the duration of the dark treatment. During exposure to light in a Wardian case the stem continued to elongate and new leaves developed. The upper part of the stem which developed in light was similar in appearance at first to the lower part of the stem which had been previously covered with black cloth. Along the entire length of some stems roots had penetrated the epidermis. In contrast to the blanched condition of the stem and basal parts of leaves, the upper parts of the leaves were dark green in color. Plants completely covered with black cloth were so badly damaged at the end of seven days that the upper part of the stem and most leaves were either badly wilted at this time or collapsed within a few hours after exposure to light. This type of injury was previously described (7, p. 228). Tomato plants which were completely covered with black cloth showed little or no swelling and proliferation characteristic for the capped plants. The plant illustrated in Figure 2 B represents the minimum of injury resulting from darkening the entire plant for one week and thereafter exposing only the lower part to light for two more weeks, the upper part of the plant being capped when the lower part was exposed to light. Most of the other plants subjected to this same combination of dark and light treatments exhibited more injury. However, it is to be noted that the upper part of

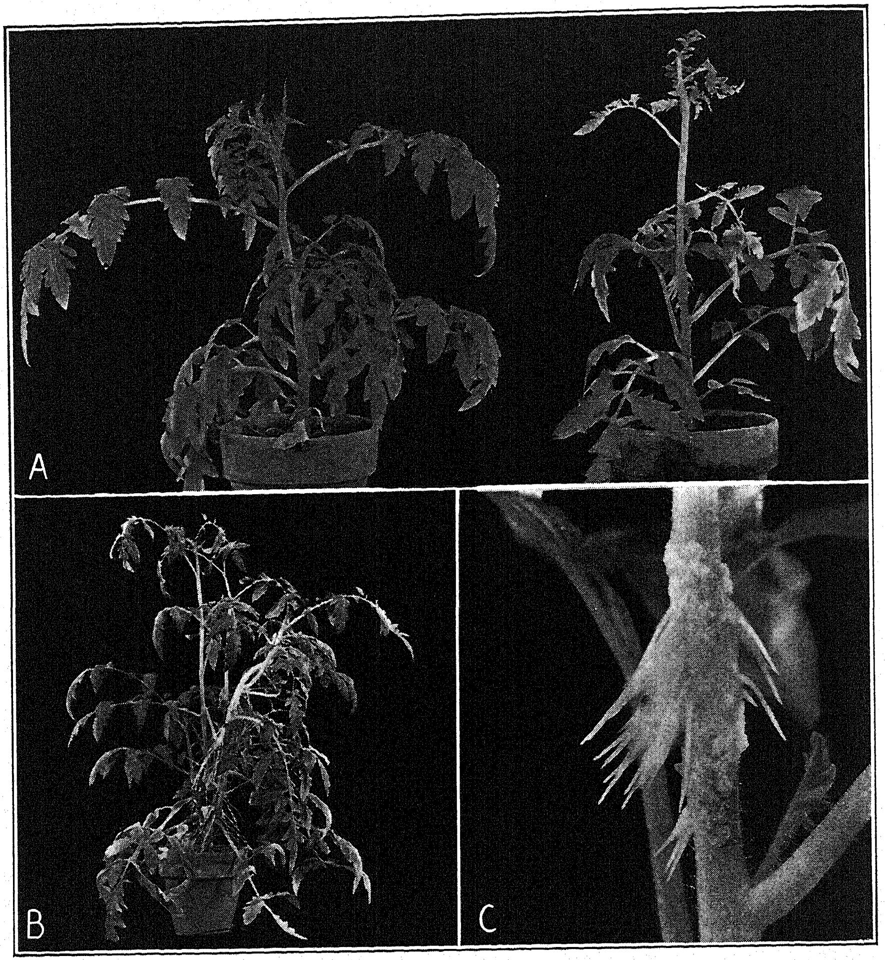

FIGURE 2. Physiological responses induced by capping the upper parts of tomato plants with black cloth. (A) Left, control not capped. Right, capped for 7 days. (Photographed two days after removal of cap.) (B) Capped 14 days. (Photographed 17 days after removal of cap.) Rooting response resembles plants treated with such growth substances as ethylene, indoleacetic acid, naphthaleneacetic acid, etc. Note pronounced growth of axillary shoots. (C) Close-up of rooting region on plant above. Shows swelling and intumescence-like proliferation characteristic of unsaturated carbon gases, indoleacetic acid, naphthaleneacetic acid, etc.

the plant shown in Figure 2 B survived continuous exposure to darkness for a period of 21 days and at this time had an intact growing tip. The upper parts of all completely darkened plants were dead at the end of 21 days. Effects of darkness were previously described also for other species (7).

Stems which had proliferated as a result of capping were generally of larger diameter than stems on control plants. This was particularly true of the region near the growing tip. Whereas the stems of control plants were decreasingly smaller in diameter toward the tip, the proliferated stems of capped plants were of nearly the same diameter to within one inch or less of the growing tip.

Root formation. Dark treatment of the tomato plants caused roots to emerge from the main stem, from the stems of axillary shoots which had developed on some of the plants during the dark period, and from the petioles of one or more leaves under the cap. Roots usually emerged first from the basal portion of the etiolated stem (Fig. 1 B) and later from regions above. The roots shown in Figure 2 C represent the elongation which occurred during the two days the plants remained in a Wardian case and subsequent to removal of the black caps. After the capped region was exposed to light, many roots emerged from various parts of the etiolated stem.

The type of root formation illustrated in Figure 2 is characteristic of the root formation induced by applied growth substances (11). It is to be observed, also, that the swelling and proliferation associated with rooting in this case (Fig. 2, B and C) is characteristic of that resulting from treatment with gases such as ethylene (6) and other growth substances (11).

Using essentially the methods of Smith (4) and Gardner (3), stems of potted tomato plants were wrapped with black cloth for a distance of about one and one-half inches immediately below the growing tip, but so done as not to prevent continued growth of the shoot. Seven days later the cloth was removed and cuttings were made by severing the shoot near the base of the etiolated stem region. These cuttings, together with those made from control plants not wrapped, were planted in sand on the greenhouse bench where they remained for four days. The earlier rooting (after only four days in sand) of the cuttings made from wrapped shoots is illustrated in Figure 1 B.

Influence on growth correlations. The growth of axillary shoots on plants having the region of elongation covered with black cloth was quantitatively and qualitatively different from that on control plants. Not only did a larger number of axillary buds start to grow on the treated plants, but they continued growing, as illustrated in Figure 2 B, for periods up to five weeks. In contrast, there was no appreciable growth of axillary buds on control plants even though several shoots started. Thus the correlations normally associated with apical dominance were not exhibited by plants subjected to the dark treatment for six days or longer.

Although axillary shoots generally grew from the exposed stem below the covered region, on plants capped for two weeks some shoots grew also from the etiolated stem under the cap. Roots emerged from the basal part of these etiolated axillary shoots as well as from the etiolated portion of the main stem. Continued growth of several axillary shoots occurred even though the main shoot was intact and growing. Noticeable swelling and proliferation of the main stem continued after the black cloth was re-

TABLE I

INFLUENCE ON AXILLARY SHOOT GROWTH RESULTING FROM COVERING PARTS OF TOMATO PLANTS WITH BLACK CLOTH

Number days covered	Length of shoot (mm.)**					Length of longest leaf (mm.)					Class totals
	Location (node) below cap				Group totals	Location (node) below cap				Group totals	
	1st	2nd	3rd	4th		1st	2nd	3rd	4th		
A. Tops of plants capped with black cloth*											
7	5	12	7	2	26	5	20	13	3	41	162
7	6	20	10	3	39	9	23	20	4	56	
6	3	5	6	4	18	3	4	15	8	30	82
6	4	5	4	1	14	3	8	7	2	20	
3	0	0	1	0	1	0	0	0	0	0	23
3	0	0	2	3	5	1	6	3	7	17	
0†	0	2	3	3	8	0	3	3	4	10	32
0†	0	1	2	3	6	0	2	1	5	8	
Column totals	20	52	41	24	117	23	75	69	40	182	
B. Upper part of stem wrapped with black cloth††											
7	4	5	3	6	18	2	9	2	9	22	85
7	5	5	6	5	21	3	6	9	6	24	
6	6	4	6	10	26	3	5	9	28	45	110
6	7	5	2	4	18	8	5	3	5	21	
3	0	0	2	3	5	0	2	2	7	11	28
3	0	1	2	1	4	1	1	1	5	8	
0‡	1	2	3	3	9	1	1	4	5	11	45
0‡	1	5	3	2	11	1	8	3	2	14	
Column totals	24	30	32	40	112	19	42	37	76	156	

* Leaves and upper part of stem covered.
** Measured from base of shoot to growing point.
† Transferred from bench to Wardian case at end of sixth day.
†† Stem wrapped for a distance of 5 cm. below growing tip.
‡ Left on greenhouse bench.

moved. The longer the duration of dark treatment, the more pronounced was this type of continued swelling and proliferation, provided the growing tip was not killed. Whereas the stem and the basal parts of petioles remained blanched, the leaflets and upper part of the petioles were dark green. All of these responses (swelling, proliferation, and root formation) resembled very closely those previously described as being induced by concentrations of applied growth substances considerably above the threshold values.

Data recorded in Table I show the influence of duration of the dark treatment on axillary shoot growth. It required more than three days of darkness to cause any noticeable difference in axillary shoot growth below the covered region. Plants covered for six or seven days produced noticeably larger axillary shoots as compared with control plants which were not covered (Group totals, Table I). In the case of capped plants, axillary shoot growth was most pronounced at the second and third nodes below the covered region (Column totals, Part A, Table I). Plants with part of the stem zone covered (Part B, Table I) did not exhibit a similar preferential growth (Column totals, Part B). However, the total growth performance of axillary shoots was greater for both types of dark treatment than on controls (Class totals, Table I). The measurements appearing in Table I were made seven days after the experiment was started and hence represent the responses which occurred during the earlier stages of the capping tests.

Other tests in which some of the control plants were decapitated showed that the growth of axillary shoots was more rapid at all stages as compared with the axillary shoot growth on capped plants. Thus the effect of capping was not quantitatively the same as that resulting from mechanical removal of the growing tip by decapitation. These results indicate that the mechanism regulating correlative inhibition was modified (presumably by disappearance or inactivation of the natural inhibiting hormone) but not entirely disrupted by the capping procedure.

DISCUSSION

Results obtained with capping were similar to those described for tomato plants subjected to the gravity stimulus (9). However, some of the effects of capping were more pronounced than those caused by the gravity stimulus. The point of special interest in both types of experiments is that characteristic growth substance responses were induced by an initial stimulus other than a chemical substance. Insofar as the initiation of the various well-known physiological responses is concerned, it appears that in these tests the stimulus furnished by the capping procedure is essentially the same as the stimulus furnished by applying such substances as indoleacetic acid, indolebutyric acid, naphthaleneacetic acid, phenylacetic acid, and ethylene.

The principal limiting factor in these tests was the duration of the dark period. Optimum effects were usually obtained when the black caps were left on for about ten days. Longer periods, particularly 14 days or more, caused permanent injury to the lowermost leaves under the cap and in some cases to the growing tip. Periods shorter than ten days caused less pronounced responses. The importance of exposing part of the plant to normal daylight during the dark period was evidenced by comparative results with completely darkened plants. In the latter case serious damage to or death of the aerial parts occurred after two weeks which is in accordance with the results perviously reported (7). Such results indicate that substances important for survival of the capped aerial parts were furnished by the lower parts exposed to light and were presumably transported to the capped parts above. However, it is a matter of interest that the capping treatment should produce an effect which indicated disappearance or inactivation of the axillary bud-inhibiting hormone and at the same time produce effects which indicated that a marked increase in hormone production had occurred. The separation of bud-inhibiting effects from growth promotion and organization effects warrants further study.

Although certain effects of etiolation have been described by others (4, 3), most of the work has dealt with root formation. In contrast, the present results show that capping tomato plants induced essentially all responses characteristic for applied growth substances—that is, curvatures, swelling, proliferation (including the formation of intumescences), inhibition of growth, initiation of adventitious roots, and disturbance of apical dominance correlations. These typical responses are similar to those induced by the most active growth substances (e.g. indoleacetic acid, indolebutyric acid, naphthaleneacetic acid, and ethylene). Relatively high concentrations of applied growth substances are required to induce rooting on attached tomato leaves equivalent to the rooting caused by merely capping the upper part of the tomato for a period of ten days. For example, the threshold value for indoleacetic acid was found to be 500 p.p.m. (8, p. 340) for rooting on attached tomato leaves.

Lacking any specific knowledge of the identity of the naturally-occurring growth substances in green plants, with the exception of ethylene, no estimate can safely be given of the probable concentration or of the total quantity of natural growth substance in the capped tomato plants. On the basis of the concentration of applied growth substances such as indoleacetic acid, indolebutyric acid, naphthaleneacetic acid, etc., which would be required to induce responses similar to those described, the threshold values of 1 to 500 p.p.m. for tomato are of the order of magnitude termed by Went and Thimann as unphysiological (5). Designating any specific concentration of a growth substance as physiological or unphysiological is an assumption which has not yet been substantiated by experimental facts.

Responses of capped tomato plants are of interest not only because they were induced by an initial stimulus other than a chemical substance, but because the effects indicate that such pronounced responses were probably due to an increase in the total quantity of naturally-occurring growth substances. The present results indicate that the influence of gravity, light (including the effect of darkness), and applied growth substances (indoleacetic acid, naphthaleneacetic acid, etc.) all act essentially alike in furnishing the initial stimulus which starts the action or actions finally resulting in an increase in production of natural hormones.

In view of the results described here as well as those relating to the responses of plants to vapors of many different substances (10), explanations for the action of natural and applied growth substances based on some of the earlier theories and assumptions are not satisfactory and are not in agreement with known facts. The similarities previously discussed (1) between the effects of such gases as ethylene and carbon monoxide and the effects of many active growth substances become more striking as additional facts accumulate. Sufficient consideration seems not to have been given to the limitations attending any given method of applying growth substance preparations to test objects.

SUMMARY

Capping the upper part of the tomato plant with black cloth for periods of 3 to 14 days induced physiological responses such as epinasty of leaves, swelling, proliferation (including the formation of intumescences), inhibition of growth, initiation of adventitious roots, and disturbance of apical dominance correlations. These typical responses are similar to those induced by treatment with applied growth substances such as indoleacetic acid, indolebutyric acid, naphthaleneacetic acid, and ethylene in concentrations of 1 to 500 p.p.m. The magnitude of the responses indicates that they were caused by an increase in the production of natural hormones and not entirely as a result of a redistribution of the existing hormones during the dark treatment.

Although the premature growth of axillary buds on capped plants indicated a decrease in the apical dominance effect, other responses indicated that a marked increase in hormone production had occurred. Thus, at least a partial separation of the bud-inhibiting influence from the influence of other natural hormone effects was obtained in the capped plants.

Insofar as the initiation of the various well-known physiological responses is concerned, the stimulus furnished by capping was essentially the same as the stimulus furnished by applying indoleacetic, indolebutyr-

ic, naphthaleneacetic, and phenylacetic acids, ethylene, and other active growth substances.

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STORAGE OF SOME FLOWER SEEDS

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INTRODUCTION

The rapid deterioration of many flower seeds under ordinary methods of storage has led a number of seedsmen and florists to publish notes at various times on their own personal experiences with some kinds of seeds. These notes have not usually included experimental data, but, for the most part, have simply emphasized the need for well planned experiments from which reliable storage methods could be determined. Some tests conducted by the California Federal State Seed Laboratory in which 64 samples of flower seeds representing 30 genera were stored for ten years represent extensive work to obtain necessary data on the longevity of these forms. The results obtained from this experiment have been reported by Goss (14). Although this report contains much information of vital importance, it does not shed any light on the possible extension of life under special storage conditions since all of the seeds were merely placed in paper envelopes and stored in a tin box in the seed laboratory at Sacramento.

Data now available on many different seed forms demonstrate conclusively that seeds whose life span supposedly does not exceed three years can, by proper storage, be kept in a viable state for three to fifteen years or even longer. The longevity of any particular kind of seed thus becomes a function of the storage conditions under which it has been kept rather than its actual age.

It is the purpose of this paper to present evidence showing that certain flower seeds may have their longevity increased many-fold by controlling the conditions under which they are stored. Storage conditions which promote deterioration will also be presented. The comparative effects of moisture content of the seeds together with the influences of temperature and surrounding gases will be discussed.

METHODS

In the experiments presented below, vitality tests were made both on moist filter paper or in granulated peat moss and in soil in the greenhouse. In the former case ovens at controlled temperatures were used for all of the seeds except dandelion and aster, both of which were germinated at room temperature. The controlled temperatures used were as follows: verbena, daily alternation of 20° to 30° C.; regal lily and venidium, daily alternation of 15° to 30° C.; sweet pea and pansy, constant 20° C.; delphinium, constant 15° C. In cases of daily alternations of temperature the cultures were left at the lower temperature for 16 hours and at the higher temperature for 8 hours daily. Oven tests of aster, dandelion, verbena, venidium, and delphinium were made in Petri dishes on moist filter paper, while seeds of regal lily and sweet pea were mixed with moist granulated peat moss and placed in bottles. The soil used for greenhouse plantings consisted of a mixture of sand, sod soil, and granulated peat moss in equal parts. Germination counts on moist filter paper or in moist granulated peat moss were made as soon as the radicle emerged from the seed. Seedlings in soil were counted as the shoot appeared above ground. Duplicates of 100 seeds each were used for each test. The figures presented in the tables are averages of these duplicates. Thus 400 seeds were used for each vitality test.

Moisture determinations were made at first by drying seed lots, usually in duplicates of approximately 2 grams each, in a vacuum oven at 80° C. to a constant weight and then calculating water loss on the basis of dry weight. In later tests a drying period of 48 hours was used, this period having been shown to be sufficient by a series of tests in which different periods were used. If reduced moisture contents were desired, seeds with a known percentage of moisture were weighed and placed over calcium oxide in desiccators and allowed to remain until the approximate desired water loss was obtained. Samples were then taken for actual moisture determinations as described above and the remaining seeds stored in any manner desired.

The length of time the seeds needed to remain over calcium oxide to reduce the amount of moisture present depended upon the type of seed, the moisture content desired, the size of the desiccator, and the quantity of seeds. Twenty-four hours were sufficient to reduce the moisture content of aster seeds from 7.9 per cent to 6.7 per cent when they were spread in a rather thin layer. Seventy-two hours over calcium oxide brought about a further reduction of moisture in these seeds to 4.6 per cent. On the other hand, sweet pea seeds with an initial moisture content of 10.9 per cent dried for 20 days still retained 9.6 per cent water.

Three storage temperatures, room temperature, 5° C., and approximately −5° C., were used. The laboratory with seasonal temperature and humidity fluctuations was used for room temperature storage. Although the temperature of the 5° C. room was fairly constant, varying only ±0.5° C., the high humidity there figured prominently in the deterioration of the seeds. Below-freezing storage was in a room with a temperature which varied from −2° to −7° C., with −5° C. as the temperature which prevailed the greater portion of the time. The humidity of this room was not so great as that at 5° C.

Both open and sealed containers were used. Bottles with stoppers coated with sealing wax, tin cans with tight-fitting lids sealed with sealing wax, and small glass tubes hermetically sealed were used. In the last instance, the tubes containing the seeds were drawn out to a very small bore and allowed to cool. Sealing of some was then completed so that the tubes contained air. Other tubes were attached to the suction line of a vacuum pump with a graduated T-tube extending into a reservoir of mercury between the vial and the suction line. When the air was withdrawn so that the mercury had risen to 750 mm. in the T-tube, the vial was sealed while still attached to the suction line. A partial vacuum was thus obtained in the storage vial. Vacuum sealing was used for dandelion, aster, and verbena.

New crop seeds of dandelion, improved thick leaved No. 415 (*Taraxacum officinale* Weber), aster, Queen of the Market (*Callistephus chinensis* Nees), and *Verbena teucrioides* Gill. & Hook., Royal Bouquet mixed, were obtained from W. Atlee Burpee Seed Company, Philadelphia, Pennsylvania, in October, 1935. Winter-flowering sweet pea (*Lathyrus*), pansy, Giant Golden Queen (*Viola*), and *Venidium* seeds used were furnished by George Ball, West Chicago, Illinois, and were received in January, 1935. Seeds of the regal lily (*Lilium regale* Wilson) collected in Yonkers, New York, in 1931 were sent to the seed laboratory in March, 1932. The *Delphinium* seeds were obtained from Vaughan Seed Company, New York, N. Y., in December, 1926.

EXPERIMENTAL RESULTS

DANDELION

Dandelion, a wild flower which is usually classed as a weed, has considerable economic importance. The leaves are used as greens or salad and the seeds are often sold in large quantities. Seedsmen report difficulty in keeping these seeds viable for longer than a year in certain localities.

Storage tests designed to show moisture, temperature, and vacuum effects on vitality of these seeds were started. At the time the seeds were received, they contained 7.9 per cent moisture. One lot of seed was stored with this moisture content while other lots were dried in desiccators over calcium oxide and their moisture contents reduced to 6.2, 5.0, and 3.9 per cent before storage. Individual seed lots were then stored in small glass tubes hermetically sealed in air and in vacuum at room temperature, 5° C., and −5° C. Other lots with the same moisture contents were placed in tin cans with tight-fitting lids sealed with sealing wax. In the latter case each can was opened and samples taken out for testing every six months, after which the cans were re-sealed. In the glass tubes, however, each sample was sealed separately. A comparison of two sealing methods was thus obtained. The glass tubes also permitted the study of vacuum effects, which could not be obtained when tin cans were used. Tests were made in duplicates of 100 seeds each, both on moist filter paper at room temperature and in soil in the greenhouse. Tests up to and including the three-year period have been made and are shown in Table I. The low seedling production obtained in the greenhouse planting after two years of storage was due to the damage to the seeds by ants.

Open storage of air-dry seeds at room temperature resulted in a decrease of vitality so that the seeds gave only 37 per cent germination after one and one-half years of storage and were worthless for planting after two years. A similar loss in vitality was evidenced for the open lot at 5° C. This may have been due, at least in part, to the high humidity of the 5° C. room resulting in high water content of seeds. On the other hand, open storage at −5° C. kept the seeds perfectly for a period of three years (Fig. 1).

Sealing air-dry seeds favored the retention of vitality so that after three years of storage, those from room temperature still germinated 47 per cent, and those from both 5° C. and −5° C. showed full germination capacity.

When the germination of seeds with reduced moisture contents was determined, the storage temperature effects became less marked as the moisture content was lowered so that with 3.9 per cent moisture seeds showed little difference in vitality after three years at room temperature, 5° C., or −5° C. This may be seen from Table I and is pictured graphically in Figure 1. A wide divergence existed in open storage between −5° C. on the one hand and 5° C. due to the high humidity, and room temperature due to the high temperature on the other hand. This divergence became marked after one and one-half years of storage (Fig. 1). If air-dry seeds were sealed, 5° C. and −5° C. were equally favorable for storage and the curve for retention of vitality at room temperature diverged less widely from those for the low temperatures. Differences in temperature effects became still less noticeable when about 1 per cent of the moisture was removed from the seeds before storage, and when a larger amount of the moisture was removed leaving only 3.9 per cent in the stored seeds, it was not necessary to use a cold room to keep them viable for a period of three years.

The comparative effects of sealing in glass tubes versus sealing in tin cans when dandelion seeds were stored at room temperature are shown in Figure 2. It should be kept in mind that the tin cans were opened at the end of each six-month period and a seed sample was removed for testing, after which the lid was replaced and the can re-sealed. Also there might have been a leakage of air through the seal on these cans. Each glass vial, on the other hand, contained enough seeds for one test only. As the moisture present in the seeds at the time of storage was reduced, the deleterious effect of opening the storage container at intervals was decreased, so that with a moisture content of 3.9 per cent, seeds lived three years regardless of the storage container. Seeds containing 7.9 per cent moisture and sealed in tin cans fell rapidly in vitality when stored at room temperature for longer than one year. After one and one-half years these seeds gave only 4 per cent germination. Removing about 1 per cent of the water resulted in prolonging their longevity so that 45 per cent were

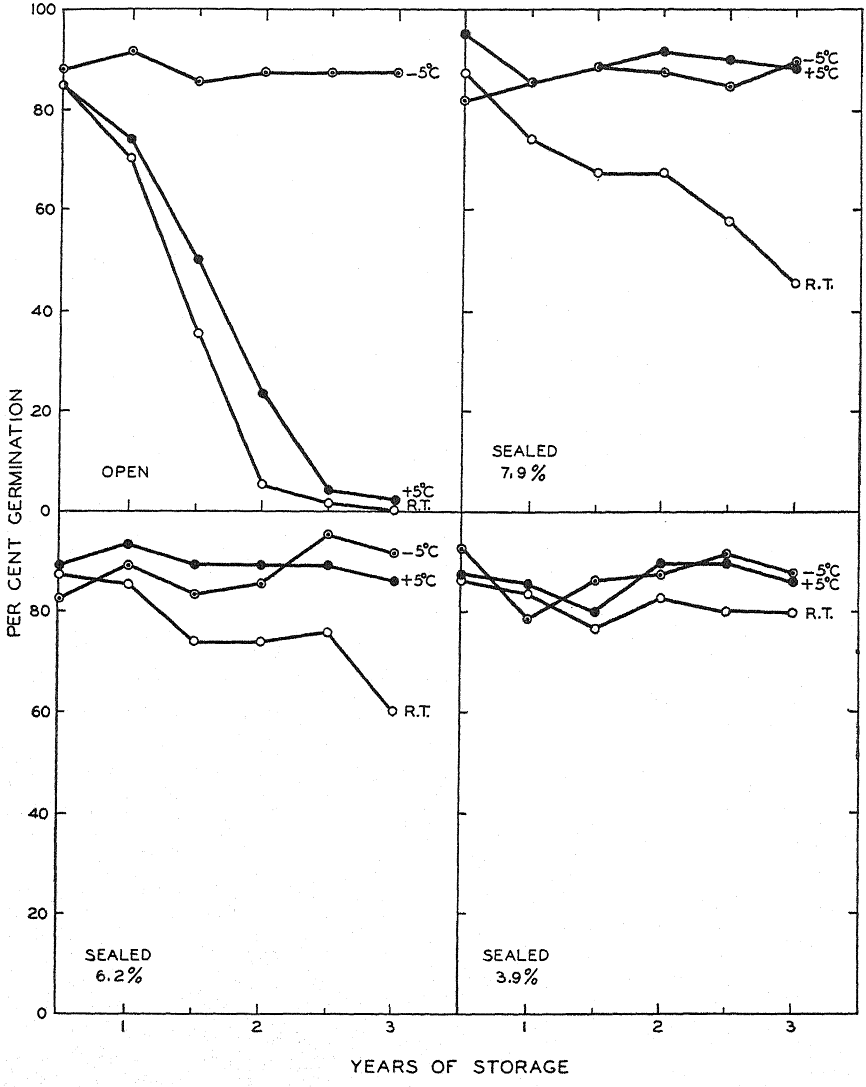

FIGURE 1. Germination of dandelion seeds on moist filter paper after storage for various periods at room temperature (R.T.), +5° C., and −5° C. in open containers (upper left) or sealed in glass tubes (upper right and lower half) with moisture contents of 7.9, 6.2, and 3.9 per cent. "Open" lots moisture at start was 7.9 per cent.

still viable after one and one-half years and a further reduction in moisture content to 5.0 per cent permitted 48 per cent of the seeds to germinate after two years of storage. If the seeds were dried to 3.9 per cent moisture before storing, there was a distinct prolongation of life at room temperature sealed in tin cans, 62 per cent of these seeds germinating after three years of storage. Here is shown the striking effect of drying on retention of vitality at ordinary room temperature.

Moisture determinations made on dandelion seeds after three years and five months of storage at room temperature showed that the moisture contents of the dried seeds in tin cans had increased greatly over that present at the time of storage, while seeds which contained 7.9 per cent moisture at the beginning of the experiment had dried somewhat. Seeds having moisture contents of 7.9, 6.2, 5.0, and 3.9 per cent at the time of storage showed 6.7, 6.3, 6.3, and 5.6 per cent respectively after more than three years of storage.

These figures, together with the behavior of the seeds as shown in Figure 2, indicate that dandelion seeds with a "critical" moisture content between 6 and 8 per cent deteriorated much more rapidly when fluctuation in moisture was introduced, as in the repeated opening of the tin cans, than when the moisture remained constant in the "critical" range, as was the case when sealed glass tubes were used. The lower the percentage of moisture at the time of storage, the longer it required for repeated openings to increase the moisture to the "critical" point where fluctuations caused rapid degeneration. Hence with increased initial drying to at least 3.9 per cent moisture the seeds remained viable for progressively longer periods. Since seeds sealed in tin cans with an initial moisture content of 3.9 per cent now contain 5.6 per cent, it is to be expected that a rapid decline in viability will take place in the near future. The beginning of such a decline may already be indicated (Fig. 2).

Moisture determinations on seeds with 7.9, 6.2, 5.0, and 3.9 per cent moisture in sealed glass vials after three years of storage showed that the initial moisture content was retained.

Seeds stored open in the laboratory contained 5.6 per cent moisture in March 1939 after storage for over three years whereas 7.9 per cent was present when they were received. Fluctuations doubtless occur throughout the storage period in open containers.

All seeds with moisture contents of less than 7.9 per cent showed no effect of a vacuum at any of the three temperatures tried. This was not surprising since sealed storage in glass tubes in air under these conditions was sufficient to maintain the germination power for three years. It will be noted, however (Table I), that the vacuum seemed to increase the speed of deterioration of seeds with 7.9 per cent moisture both at room temperature and at 5° C. storage. This effect is difficult to explain in view of vacuum effects obtained in other cases, where the tendency was to prolong the life of seeds stored under unfavorable conditions (4, 7, 15). Consequently several different storage lots were tested at both the two and one-

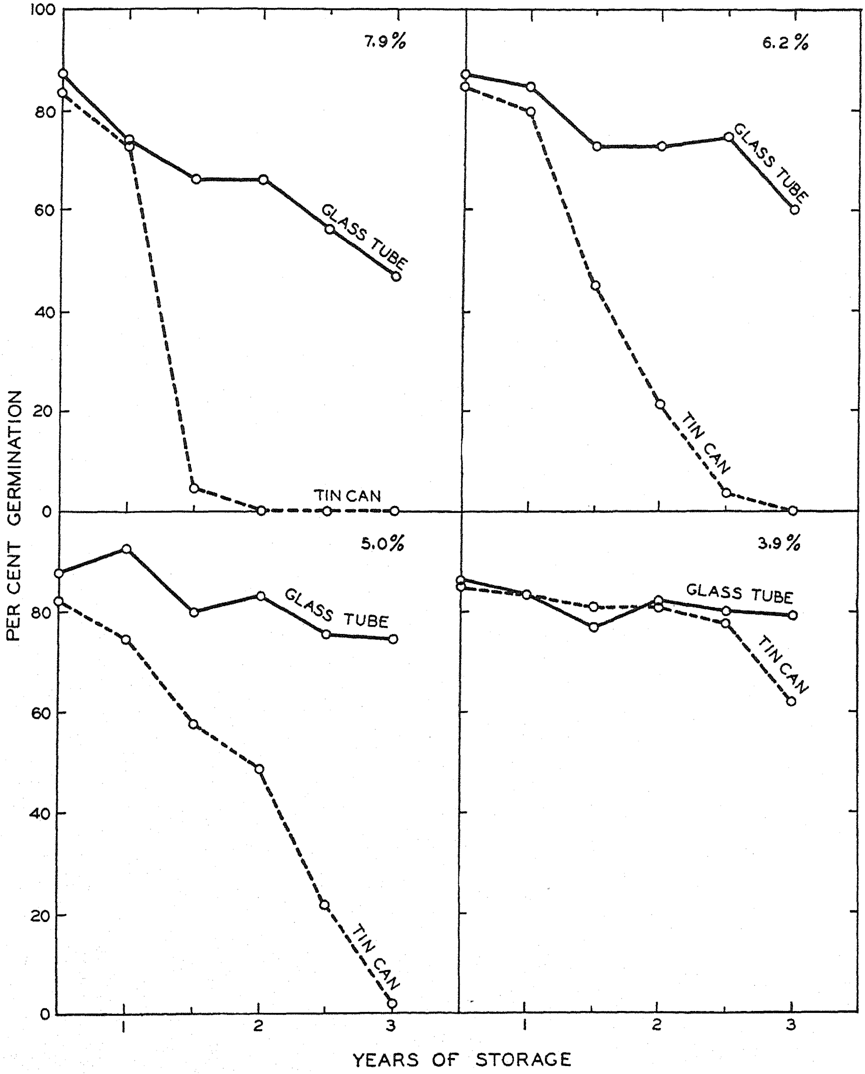

FIGURE 2. Effect of sealing dandelion seeds with moisture contents of 7.9, 6.2, 5.0, and 3.9 per cent on their vitality after storage for various periods at room temperature. Glass tubes were not opened from the time of sealing until the vitality tests were made. Tin cans were opened at intervals of six months for removing samples and then re-sealed. Germination on moist filter paper.

TABLE I

DANDELION—GERMINATION ON MOIST FILTER PAPER AT ROOM TEMPERATURE (RT) AND SEEDLING PRODUCTION IN SOIL IN THE GREENHOUSE (GH) AFTER STORAGE. DUPLICATES OF 100 SEEDS USED FOR EACH TEST*

Storage conditions			Per cent after storage for years											
Moisture content, %	Open or sealed	Temperature	1/2		1		1-1/2		2		2-1/2		3	
			RT	GH	RT	GH	RT	GH	RT	GH	RT	GH	RT	GH
Containers, glass tubes														
7.9	Open	Room	84	81	69	40	37	21	5	1	0	0	0	1
		5° C.	84	71	73	58	50	41	24	3	4	2	2	2
		−5° C.	88	83	91**	80	85	84	87	41	87	69	87	69
	Sealed in air	Room	87	80	73	72	66	51	66	21	56	35	47	21
		5° C.	95	87	85	78	88	80	91	39	90	63	88	71
		−5° C.	81	87	85	82	88	81	87	34	84	75	90	78
	Sealed in vacuum	Room	91	85	73	23	30	17	88	50	89	63	3	1
		5° C.	94	88	86	80	87	80	85	38	11	4	30	12
		−5° C.	87	82	95	80	88	81	92	39	90	81	93	70
6.2	Sealed in air	Room	87	62	85	68	73	59	73	29	75	42	60	39
		5° C.	89	73	93	78	89	82	89	41	89	74	86	73
		−5° C.	82	81	89	73	83	78	84	50	95	73	91	72
	Sealed in vacuum	Room	90	73	93	82	89	85	93	27	89	70	83	66
		5° C.	91	83	84	83	90	79	94	52	93	78	83	58
		−5° C.	92	86	87	77	90	78	86	46	89	77	92	85
5.0	Sealed in air	Room	87	81	92	72	80	69	83	23	75	52	74	44
		5° C.	87	79	90	80	90	84	88	25	89	75	91	70
		−5° C.	89	87	89	85	80	75	90	59	93	75	84	77
	Sealed in vacuum	Room	90	87	84	79	80	68	89	39	89	71	82	—
		5° C.	84	87	74	83	91	77	46	12	89	84	87	80
		−5° C.	87	89	90	80	89	73	91	50	87	70	88	65
3.9	Sealed in air	Room	86	69	83	77	76	82	82	34	80	56	79	71
		5° C.	87	85	85	85	80	87	89	25	89	68	85	70
		−5° C.	92	77	78	71	86	86	87	59	91	73	87	77
	Sealed in vacuum	Room	84	88	86	69	83	78	35	6	87	76	88	64
		5° C.	86	77	83	80	91	86	91	18	89	76	92	74
		−5° C.	87	82	90	75	88	82	85	58	91	74	86	71
Containers, tin cans														
7.9	Sealed in air	Room	83	73	72	10	4	2	0	0	0	0	0	0
		5° C.	92	82	83	69	88	80	86	19	90	73	82	69
		−5° C.	94	82	88	76	90	75	85	28	92	68	90	75
6.2		Room	85	78	80	52	45	18	22	0	3	1	0	1
		5° C.	93	85	92	82	91	82	93	18	86	78	85	74
		−5° C.	89	82	92	79	87	76	88	35	92	76	92	83
5.0		Room	82	76	74	47	57	48	48	9	22	8	2	4
		5° C.	86	84	91	82	81	80	92	30	88	75	85	78
		−5° C.	84	82	82**	88	91	85	90	52	95	77	87	81
3.9		Room	85	82	83	69	81	67	81	37	77	42	62	46
		5° C.	87	83	87	83	89	82	92	48	90	74	85	68
		−5° C.	92	80	86	86	89	88	90	35	92	76	90	77

* Fresh seeds gave 91% germination on moist filter paper at room temperature and 83% seedling production in soil in the greenhouse.

** One lot of 100 seeds.

half and the three-year periods in order to reduce the error of sampling but the effect persisted. Aster and verbena reported below did not behave in this manner.

From the results obtained in these tests, it seems that dandelion seeds can be stored safely at room temperature for three years if the moisture content is reduced to at least 5 per cent and if the containers are not opened until the end of the period. If the containers are opened as often as every six months at Yonkers, New York, it is necessary to reduce the moisture content to as low as 3.9 per cent at the beginning of the storage period in order to retain vitality for three years. Sealed storage proved superior to open storage regardless of moisture content (up to 7.9 per cent) when room temperature or 5° C. was used. A temperature of $-5°$ C. kept the seeds viable for three years regardless of sealing or moisture content.

ASTER

Seeds were stored in the same manner as that described above for dandelion. Again two types of sealed storage were used. In one case glass tubes hermetically sealed were not opened from the beginning of the experiment until the vitality tests were made while tin cans sealed with sealing wax were opened at the time of each test and re-sealed.

Air-dry aster seed contained 7.9 per cent moisture at the beginning of the tests. One lot was stored air-dry while two other lots were stored with reduced moisture contents of 6.7 and 4.6 per cent.

Results of vitality tests made on these seeds after storage for one-half, one, one and one-half, two, two and one-half, and three years indicated effects similar to those reported above for dandelion. Many of the seedlings produced in the greenhouse from seeds stored one year were infected with damping-off fungus, which accounts for the low figures obtained. This was also true for verbena planted at the same time.

To maintain viability of these seeds in open containers for periods longer than one and one-half years, it was necessary to use a temperature below freezing under the conditions of this experiment (Table II). There was some indication that deterioration was more rapid in open containers in a 5° C. room with a saturated atmosphere than at room temperature where the humidity was not so high. However, the rate of decline in germination power in these two storage conditions was similar, the seeds being worthless in both cases after storage periods of longer than one and one-half years.

If air-dry seeds were in sealed containers, the length of life at both room temperature and 5° C. was prolonged to at least three years, when the last test was made.

Again as for dandelion seeds a deleterious effect was evidenced from the opening of the room-temperature storage containers before the end of the test period (Table II). For example, seeds with 7.9 per cent moisture stored in tin cans produced only occasional seedlings at the end of the

TABLE II

ASTER—GERMINATION ON MOIST FILTER PAPER AT ROOM TEMPERATURE (RT) AND SEEDLING PRODUCTION IN SOIL IN THE GREENHOUSE (GH) AFTER STORAGE. DUPLICATES OF 100 SEEDS EACH USED FOR EACH TEST*

Storage conditions			Per cent after storage for years											
Moisture content, %	Open or sealed	Temperature	1/2		1		1-1/2		2		2-1/2		3	
			RT	GH	RT	GH	RT	GH	RT	GH	RT	GH	RT	GH
Containers, glass tubes														
7.9	Open	Room	86	70	83	22	71	63	3	32	15	1	0	0
		5° C.	72	53	64	22	46	29	4	16	0	12	0	0
		− 5° C.	87	75	86	50	82	74	72	80	82	78	86	62
	Sealed in air	Room	84	74	90	45	78	54	51	74	63	48	79	31
		5° C.	86	80	87	42	80	54	76	83	85	69	87	74
		− 5° C.	83	77	87	49	85	73	71	84	87	67	87	80
	Sealed in vacuum	Room	84	79	90	67	84	78	61	91	80	61	87	43
		5° C.	83	82	87	54	84	80	79	87	84	78	89	69
		− 5° C.	80	76	89	49	81	71	61	81	91	66	92	66
6.7	Sealed in air	Room	86	73	85	42	74	69	64	70	83	61	78	57
		5° C.	85	79	92	44	83	81	69	89	85	79	89	67
		− 5° C.	85	84	73	55	85	71	56	86	80	75	89	77
	Sealed in vacuum	Room	87	78	89	60	79	73	54	81	62	59	82	66
		5° C.	79	84	87	50	68	90	74	83	86	69	85	81
		− 5° C.	87	84	91	54	82	77	61	86	85	74	90	71
4.6	Sealed in air	Room	83	81	84	64	81	67	47	79	78	69	85	69
		5° C.	78	74	88	70	87	74	67	87	83	70	83	76
		− 5° C.	88	81	86	35	89	78	69	91	89	80	89	80
	Sealed in vacuum	Room	87	74	92	52	83	74	42	91	87	83	88	75
		5° C.	85	84	84	61	85	86	63	89	80	78	92	80
		− 5° C.	88	76	87	56	81	79	56	89	87	82	89	72
Containers, tin cans														
7.9	Sealed in air	Room	85	75	83	21	60	48	0	9	0	0	0	0
		5° C.	80	71	91	45	84	63	56	89	87	71	89	72
		− 5° C.	85	57	87	42	87	64	68	86	85	72	91	72
6.7		Room	84	72	83	24	78	69	35	55	33	10	9	1
		5° C.	88	70	90	34	80	70	71	92	87	80	93	76
		− 5° C.	88	75	88	36	79	68	71	92	86	68	92	64
4.6		Room	89	67	90	34	78	79	64	78	75	53	76	75
		5° C.	87	64	91	40	83	71	68	90	84	74	91	78
		− 5° C.	90	75	87	43	85	68	66	82	84	70	90	79

* Fresh seeds gave 88% germination on moist filter paper at room temperature and 67% seedling production in soil in the greenhouse.

two-year storage period while those sealed in glass tubes still gave 51 per cent germination on moist filter paper and 74 per cent seedling production in soil in the greenhouse. Seeds with moisture reduced to 6.7 per cent fared somewhat better in tin-can storage but at the end of the two and one-half-year period at room temperature gave only 33 per cent germination on moist filter paper and produced 10 per cent seedlings in the greenhouse, as opposed to 83 and 61 per cent for seeds from glass tubes.

If the moisture content was reduced to 4.6 per cent before the seeds were stored, the harmful effect of repeated opening of the storage container was still not apparent at the end of three years. This is in contrast to storage effects of dandelion seeds with 5.0 per cent moisture, which showed a complete loss of vitality when stored in tin cans for three years. These results indicate a possible higher "critical" moisture content for aster than for dandelion. This would mean that aster seeds require less drying for safe storage for a given length of time at room temperature than do dandelion seeds.

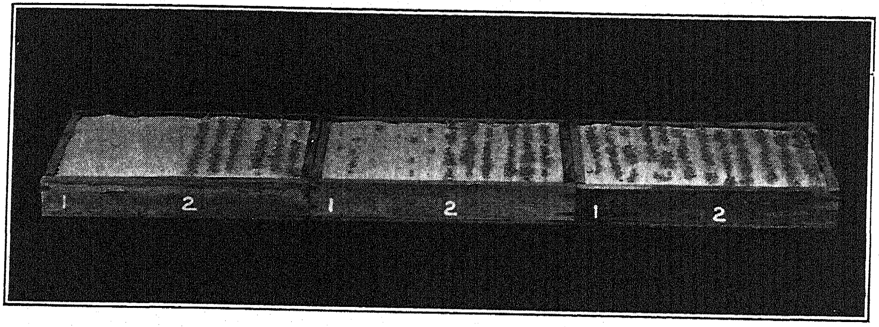

FIGURE 3. Seedling production from aster seeds stored two and one-half years sealed in tin cans at (1) room temperature, and (2) +5° C. Flats, left to right: seeds contained 7.9, 6.7 and 4.6 per cent moisture at time of storage.

Moisture determinations made on aster seeds stored in sealed tin cans at room temperature for over three years showed those with initial moisture contents of 7.9, 6.7, and 4.6 per cent to contain 7.2, 6.3, and 5.7 per cent. Those in open storage contained 5.4 per cent moisture.

The comparative effects of different moisture contents and of room temperature and 5° C. storage for two and one-half years are shown in Figure 3. Here the beneficial effect of drying the seeds to be stored in tin cans at room temperature is again evident, as are also the advantages of 5° C. over the laboratory as a storage temperature.

Aster seeds represented by six samples in the tests reported by Goss (14) all failed to germinate after the third year. The present report indicates that their longevity can be extended beyond that period.

VERBENA

Verbena seeds were stored in the same manner as those of dandelion and aster. Air-dry seeds as they were received contained only 6.2 per cent moisture which in two cases was reduced to 5.8 and 4.2 per cent. Verbena differed from aster and dandelion in that the initial tests revealed a low germination capacity. This fact did not alter the rate of loss of germination capacity, however, which was essentially the same as for the aster and dandelion, both of which had high original germination capacities. In fact, seeds in open storage both at room temperature and in the humid 5° C. room kept longer than either aster or dandelion (Tables I, II, and III). Verbena seeds still were capable of producing some seedlings after three years of storage in open containers at room temperature but only a few were able to survive for as long as two and one-half years even at 5° C. with higher humidity. These seeds as well as those of aster succumbed more readily to high humidity at low temperature than to lower humidity at higher temperature.

Reduction in moisture content under the conditions of this experiment had some effect on the seeds in sealed storage at room temperature as evidenced by the slightly lower germination after two and one-half and three years in tin cans as opposed to the same length of time in glass tubes. However, when air-dry seeds are compared this effect is not striking as in the cases of aster and dandelion. It should be kept in mind that air-dry seeds of verbena contained only 6.2 per cent moisture while those of both aster and dandelion contained 7.9 per cent. It should also be noted that reduction of the moisture content to approximately 6 per cent in the last two forms had a marked effect in prolonging their lives when stored in sealed tin cans at room temperature.

Sealed storage at low temperatures, both at 5° C. and −5° C., permitted the retention of vitality for three years regardless of moisture content. Verbena seeds of different lots were found by Goss (14) to be different in their behavior. Of three samples, one failed after the third year, one after the fifth, and one after the seventh.

SWEET PEA, PANSY, AND VENIDIUM

These seeds, all of the 1934 crop, were obtained in January, 1935. They were stored air-dry and with reduced moisture contents at room temperature, 5° C., and −5° C. Sealed storage of sweet pea and pansy seeds was effected in bottles with cork stoppers sealed over with sealing wax. Venidium seeds were sealed in small glass tubes as described for dandelion above.

Air-dry sweet pea seeds, received in two lots, contained 10.9 per cent moisture. Samples of lot B were dried over calcium oxide to 9.6 per cent moisture before storing at room temperature and 5° C. A limited supply

TABLE III

VERBENA—GERMINATION ON MOIST FILTER PAPER AT 20° TO 30° C. DAILY ALTERNATION AND SEEDLING PRODUCTION IN SOIL IN THE GREENHOUSE (GH) AFTER STORAGE. DUPLICATES OF 100 SEEDS EACH USED FOR EACH TEST*

Storage conditions			Per cent after storage for years											
Moisture content, %	Open or sealed	Temperature	1/2		1		1-1/2		2		2-1/2		3	
			20° to 30°	GH	20° to 30°	GH	20° to 30°	GH	20° to 30°	GH	20° to 30°	GH	20° to 30°	GH
Containers, glass tubes														
6.2	Open	Room	37	45	44	25	48	37	30	17	27	26	20	8
		5° C.	32	27	29	30	32	21	26	14	6	4	0	1
		−5° C.	38	59	50	28	42	48	46	30	34	37	47	42
	Sealed in air	Room	39	39	26	24	39	47	53	25	39	32	42	20
		5° C.	31	42	52	22	49	56	57	35	43	43	36	35
		−5° C.	41	59	59	26	40	58	47	38	46	42	35	45
	Sealed in vacuum	Room	50	36	40	15	27	38	50	24	33	26	42	36
		5° C.	34	41	60	15	35	30	49	25	25	41	39	38
		−5° C.	25	48	43	23	49	50	53	39	37	46	54	39
5.8	Sealed in air	Room	61	28	53	21	43	43	51	33	33	32	44	18
		5° C.	28	49	62	30	49	55	63	24	41	34	41	35
		−5° C.	27	48	65	23	28	44	60	41	42	44	44	29
	Sealed in vacuum	Room	36	25	45	20	29	39	49	24	38	38	40	32
		5° C.	31	48	43	22	38	57	53	34	47	51	30	—
		−5° C.	52	31	42	33	51	51	54	39	31	38	52	34
4.2	Sealed in air	Room	63	22	46	34	35	44	56	26	31	48	34	22
		5° C.	53	51	56	39	42	52	51	25	37	37	39	30
		−5° C.	27	36	54	33	48	42	49	33	43	45	34	33
	Sealed in vacuum	Room	49	28	45	28	39	43	50	22	28	37	38	32
		5° C.	24	46	36	36	41	38	58	24	44	44	53	41
		−5° C.	39	30	51	35	41	36	51	35	27	49	36	28
Containers, tin cans														
6.2	Sealed in air	Room	59	44	42	1	40	34	48	18	22	29	34	26
		5° C.	38	29	42	1	50	32	47	27	45	42	46	50
		−5° C.	37	61	50	1	54	43	52	31	32	38	43	46
5.8		Room	42	47	51	16	38	27	50	23	37	40	45	32
		5° C.	30	45	37	22	50	41	50	27	33	38	50	41
		−5° C.	45	56	40	23	38	51	55	37	36	38	56	43
4.2		Room	44	50	43	33	42	26	56	20	32	31	45	18
		5° C.	23	50	58	30	31	45	59	33	50	41	40	32
		−5° C.	32	36	54	29	41	46	66	33	33	46	38	32

* Fresh seeds gave 45% germination on moist filter paper at 20° to 30° C. daily alternation and 44% seedling production in soil in the greenhouse.

of seeds prevented extensive tests with this species. Germination tests were made by soaking the seeds overnight in water at 15° C. and then placing them in moist granulated peat moss at 20° C. or planting them in soil in the greenhouse. The results are shown in Table IV. The small seed supply made complete tests impossible but the seeds remained viable under all conditions for 13 months. Later tests up to 33 months indicated no definite effect of reduction in moisture content under the conditions of this experiment and showed that sweet peas will remain viable for that period.

Limited tests with pansy seeds indicated that open storage in a saturated atmosphere at 5° C. was harmful, the seeds being much reduced in vitality after 19 months of storage. Open storage at room temperature permitted retention of vitality, though somewhat reduced, up to 13 months when the seed supply was exhausted. Sealed storage at room temperature kept the seeds viable for 19 months but proved deleterious when the period was extended to 33 months. Under the conditions of this experiment, pansy seeds kept best at 5° C. in sealed containers. No effect on vitality was evidenced when the moisture content was reduced from 5.2 to 3.8 per cent.

Ball (1, p. 49) speaking of pansy seed stated that "Under favorable conditions its viability will extend over two years, but no responsible seedsman depends on this." He further remarked that storage conditions must be favorable in order to keep the seeds in good shape until the August after harvest. He did not describe favorable storage conditions.

Harrold (16) believed that some of the failures attributed to poor pansy seeds were really caused by high soil temperatures or hot dry atmosphere. He claimed to have shown "by actual test" that under the proper storage conditions pansy seed will maintain within 20 per cent of its fresh garden production at the end of the fourth season after harvest. He pointed out also that American-grown seeds were superior to imported seeds in viability. He presented no data to substantiate his statements and gave no description of the proper storage conditions.

Goss (14), reporting on the results of storing pansy seeds in paper envelopes in the seed laboratory at Sacramento, California, presented data which showed 45 per cent germination after five years of storage and 2.5 per cent after nine years.

The results of the present limited tests also indicated that pansy seeds may be kept viable for periods longer than two years and probably their life span may be increased several-fold by sealing seeds with approximately 5 per cent moisture at low temperature.

Venidium seeds were very poor in germination throughout the tests. Original tests gave only 25 per cent on moist filter paper at 15° to 30° C. daily alternation and 4 per cent in soil in the greenhouse. This vitality was maintained for 37 months under all the storage conditions tried. Again,

TABLE IV

GERMINATION IN THE OVENS AND SEEDLING PRODUCTION IN THE GREENHOUSE (GH) AFTER STORAGE. DUPLICATES OF 100 SEEDS EACH USED FOR EACH TEST

Storage conditions			Per cent after storage for months											
Moisture content, %	Open or sealed	Temperature	8		13		19		33		37		47	
			Oven	GH	Oven	GH	Oven	GH	Oven	GH	Oven	GH	Oven	GH
			Sweet pea A											
10.9	Open	Room	69	61	60	48	50	49	—	—	—	—	3	7
	Sealed	Room	67	64	72	58	—	—	20	5	—	—	8	4
	Open	5° C.	90	83	88	87	—	—	—	—	—	—	—	—
	Sealed	5° C.	76	60	73	54	67	70	71	30	—	—	—	—
			Sweet pea B											
10.9	Open	5° C.	93	80	98	81	—	—	—	—	—	—	—	—
9.6	Sealed	Room	68	54	65	48	37	50	43	10	—	—	—	3
	Sealed	5° C.	61	56	64	48	57	63	55	25	—	—	—	—
			Pansy A											
5.2	Open	Room	51	39	44	25	—	—	—	—	—	—	—	—
	Sealed	Room	50	40	56	35	49	31	24	3	—	—	—	—
	Open	5° C.	54	47	48	22	7	2	1	1	—	—	—	—
	Sealed	5° C.	68	56	67	38	61	40	63	44	—	—	—	—
			Pansy B											
5.2	Open	Room	37	30	37	21	—	—	—	—	—	—	—	—
	Sealed	−5° C.	63	48	68	50	58	52	—	—	—	—	—	—
3.8	Sealed	Room	54	39	55	28	39	36	22	2	—	—	—	—
	Sealed	5° C.	63	53	67	39	55	51	7	3	—	—	26	6
			Venidium											
5.2	Open	Room	48	12	28	24	42	13	43	12	35	18	41	8
	Sealed	Room	37	16	38	26	32	22	35	9	30	17	34	7
	Open	5° C.	25	3	17	12	16	3	14	6	9	1	3	0
	Sealed	5° C.	26	5	18	14	25	13	31	17	30	18	40	11
	Open	−5° C.	21	4	28	9	20	7	19	20	12	8	31	12
	Sealed	−5° C.	30	9	18	16	21	7	24	26	13	11	38	8
3.5	Sealed	Room	36	11	26	25	35	16	40	17	18	25	45	13
	Sealed	5° C.	31	8	21	14	25	7	24	7	19	13	37	8
	Sealed	−5° C.	31	8	16	16	25	7	26	16	17	13	29	7

it should be noted that there is no apparent relation between initial germination capacity and keeping quality.

REGAL LILY

Seeds used in these tests were of the 1931 crop and were received in March, 1932. Preliminary germination tests were made by mixing the seeds with moist granulated peat moss and placing the mixture in ovens at controlled temperatures. Constant temperatures of 1°, 5°, 10°, 15°, 20°, 25°, and 30° C. as well as daily alternating temperatures of 10° to 20° C., 10° to 30° C., and 15° to 30° C. were used. Ninety and ninety-two per cent germination were obtained at daily alternations of 10° to 20° C. and 15° to 30° C. Slightly lower percentages (79, 70, and 77 per cent) were obtained at constant temperatures of 15°, 20°, and 25° C. respectively. No seeds germinated at a constant temperature of 30° C. These seeds produced a seedling crop of 86 per cent when planted in the greenhouse. Greenhouse plantings were made in May or early June each year and in one case a test was made in November. Results of the greenhouse tests indicated that the temperatures at these times were favorable for seedling production.

Subsequent tests for determining viability were made in moist granulated peat moss at a daily alternation of 15° to 30° C. and in soil in the greenhouse. Germination at the controlled alternating temperature was practically complete after two weeks while a slightly longer period—up to 20 days—was required for the completion of seedling production in the greenhouse.

After completion of preliminary germination tests, moisture determinations were made preparatory to storage. Air-dry seeds were found to contain 9.9 per cent moisture. One lot of seeds was placed in a desiccator over calcium oxide for two days after which they were stored. Moisture determinations made on this second lot showed 4.5 per cent moisture. Air-dry seeds were stored open and sealed and seeds with reduced moisture content were stored sealed at room temperature, 5° C., and −5° C. Sealing was effected by using tin cans with tight-fitting lids sealed with sealing wax.

The results obtained from storage periods up to and including six years are shown in Table V. It will be noted that air-dry seeds remained fully viable at room temperature for one year but were reduced in germination capacity after two years and none was viable after three years. Sealing had no effect on the seeds under this condition. However, if part of the moisture was removed and the seeds then sealed, good germination could still be obtained after six years of storage at room temperature.

At 5° C., open storage again proved unsatisfactory for keeping seeds viable. No tests were made up to the three-year period but at this time the germination capacity was so reduced that only 16 per cent produced seedlings. Sealing was effective in keeping seeds viable at 5° C. for six years with either of the two moisture contents.

This indicates the effectiveness of 5° C. as a storage temperature. The cause of the rapid loss of vitality of seeds stored open at this temperature is to be found in the high humidity prevailing in this room. The combination of low temperature and high humidity proved only slightly superior to the higher temperature but lower humidity of the laboratory for keeping regal lily seeds. When the absorption of excess moisture was prevented by the use of air-tight containers, seeds remained viable for at least six years at 5° C. When this lower temperature was used for storage, air-dry seeds (with 9.9 per cent moisture) kept well but at the higher temperature of the laboratory it was necessary to reduce the moisture content in order to permit retention of vitality for longer than two years.

TABLE V

REGAL LILY—GERMINATION IN MOIST GRANULATED PEAT MOSS AT 15° TO 30° C. DAILY ALTERNATION AND SEEDLING PRODUCTION IN SOIL IN THE GREENHOUSE (GH) AFTER STORAGE. DUPLICATES OF 100 SEEDS EACH USED FOR EACH TEST*

Storage conditions			Per cent after storage for years													
Temperature	Moisture content, %	Open or sealed	1		2		3		4		4½		5		6	
			15° to 30°	GH	15° to 30°	GH	15° to 30°	GH	15° to 30°	GH	15° to 30°	GH	15° to 30°	GH	15° to 30°	GH
Room	9.9	Open	91	88	56	42	1	0	0	0	0	0	0	0	—	—
		Sealed	98	87	65	35	1	0	3	0	0	0	0	0	—	—
	4.5	Sealed	94	91	94	89	92	87	81	80	89	65	77	74	64	57
5° C.	9.9	Open	—	—	—	—	16	16	2	1	2	4	0	0	1	0
		Sealed	—	—	—	—	—	—	—	—	93	91	94	87	92	66
	4.5	Sealed	90	89	95	86	92	96	91	92	94	92	97	94	94	34
−5° C.	9.9	Open	92	98	90	78	92	95	96	87	94	92	95	87	95	92
		Sealed	97	91	95	87	92	92	96	94	96	95	96	90	96	92
	4.5	Sealed	89	93	93	89	91	96	91	87	93	91	95	86	95	96

* Fresh seeds gave 92% germination in moist granulated peat moss at a daily alternation of 15° to 30° C., and 86% seedling production in the greenhouse.

At −5° C. both open and sealed storage permitted the retention of vitality for at least six years. Within the limits of this experiment the moisture content of the seeds stored at a temperature below freezing had no effect on their keeping quality. Figure 4 A shows the storage temperature effects as related to open and sealed containers, while Figure 4 B shows the effect of reduction of moisture content on sealed storage at room temperature, 5° C., and −5° C.

It has been generally recommended that lily seeds be planted while they are fresh. Craig (9), however, stated that *Lilium auratum* will start better from year-old seeds than from fresh seeds. He also found that *Lilium regale* seeds stored five years at Weymouth, Massachusetts, germinated nearly 100 per cent.

Clement (8) obtained about 80 per cent germination from 1936 and 1937 crops of *L. regale* seeds planted on April 12, 1938, but he secured only 8 per cent germination from the 1935 crop treated in the same way. Thus he found that these seeds keep for two years but are worthless after three years. Neither Clement nor Craig described the storage conditions used. It is presumed, however, that open storage under ordinary room conditions of temperature and humidity were used. The results reported here are in agreement with those obtained by Clement, who probably had storage conditions in Ontario similar to open room temperature at Yonkers. A drier atmosphere could have accounted for the extension of the life of regal lily seeds to five years as reported by Craig.

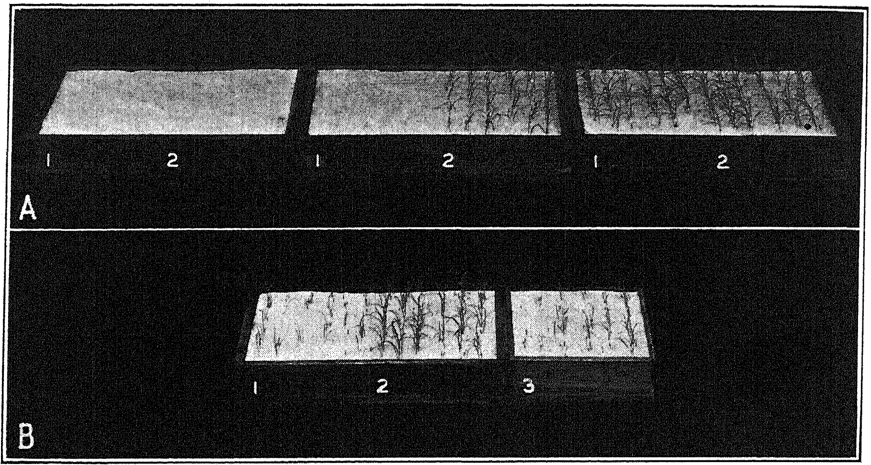

FIGURE 4. Regal lily seeds sown in flats after four and one-half years of storage. A. Flats, left to right: air-dry seeds stored at room temperature, +5° C., and −5° C. (1) Open containers. (2) Sealed containers. B. Seeds dried over calcium oxide before storage in sealed containers at various temperatures. (1) Room temperature. (2) +5° C. (3) −5° C.

If a dry cold storage room is available seeds of the regal lily can be kept fully viable for at least six years. If the atmosphere of the room is high in humidity, it is necessary to seal the seeds provided they are thoroughly air-dry (containing not more than 10 per cent moisture) unless a temperature below freezing can be had. If no cold storage chamber is available, the seeds may be kept at room temperature by reducing the moisture content to approximately 5 per cent and placing in sealed containers.

DELPHINIUM

The first report on the effect of storage conditions on the vitality of delphinium seeds was made in 1932 (2), and the details of the method may be found in that paper. The storage temperatures used were room temperature, 8° C., and −15° C. up to 1933, after which 8° C. storage lots were placed in the 5° C. room and below freezing storage was at −5° C. For convenience in comparing data reported in the first paper with that reported here, the low storage temperatures will be referred to as 8° C. and −15° C. Sealing was effected in small glass vials with cork stoppers covered with paraffin. The sealed vials were then placed in large bottles fitted with ground glass stoppers. These seeds were stored on December 26, 1926, and data are now available for tests up to and including 143 months of storage. For complete data up to 62 months of storage, one should consult the first paper (2). Some of these data have been used in presenting storage effects by means of graphs shown in Figures 5 and 6.

Annual and perennial seeds of the 1924, 1925, and 1926 crops were placed under special storage conditions in December, 1926. This means then that two-year-old, one-year-old, and fresh seeds were stored.

Figure 5 shows the effect of open and sealed storage at room temperature and 8° C. on the keeping quality of annual delphinium seeds stored for 11, 26, 35, 46, 62, 75, 87, 99, 108, 123, 133, and 143 months. The three seed crops showed similar trends throughout in their responses, despite the fact that 1924 crop seeds gave an initial germination of only 27 per cent as compared with 57 and 72 per cent for the 1925 and 1926 crops, respectively. Open storage at room temperature proved most deleterious causing rapid falling off in vitality until all seeds were worthless after 35 months of storage. When sealed containers were used, however, the decline in germination power was less rapid. Germination after 62 months in sealed containers compared favorably with that obtained after 26 months in open containers. Seeds in open storage at 8° C. fared slightly better than those in open storage at room temperature but did not keep as well as those sealed at room temperature, especially after 11 months of storage. Sealed storage at 8° C., however, served to keep these seeds viable for 143 months, in spite of the repeated openings of the storage vials.

In Figure 6 is depicted the behavior of perennial seeds under similar conditions. Here germination of seeds from −15° C. has been shown rather than those from 8° C. since the lower temperature proved best in this case. Only 1925 and 1926 crops are pictured since the 1924 crop was worthless when received in December, 1926. Again, the similar trend in behavior in spite of initial germination differences is to be noted. Open storage at −15° C. was far superior to open or sealed storage at room temperature for preserving the germination capacity of these seeds. Sealed storage at −15° C. proved best of all. Here seeds which were relatively fresh when

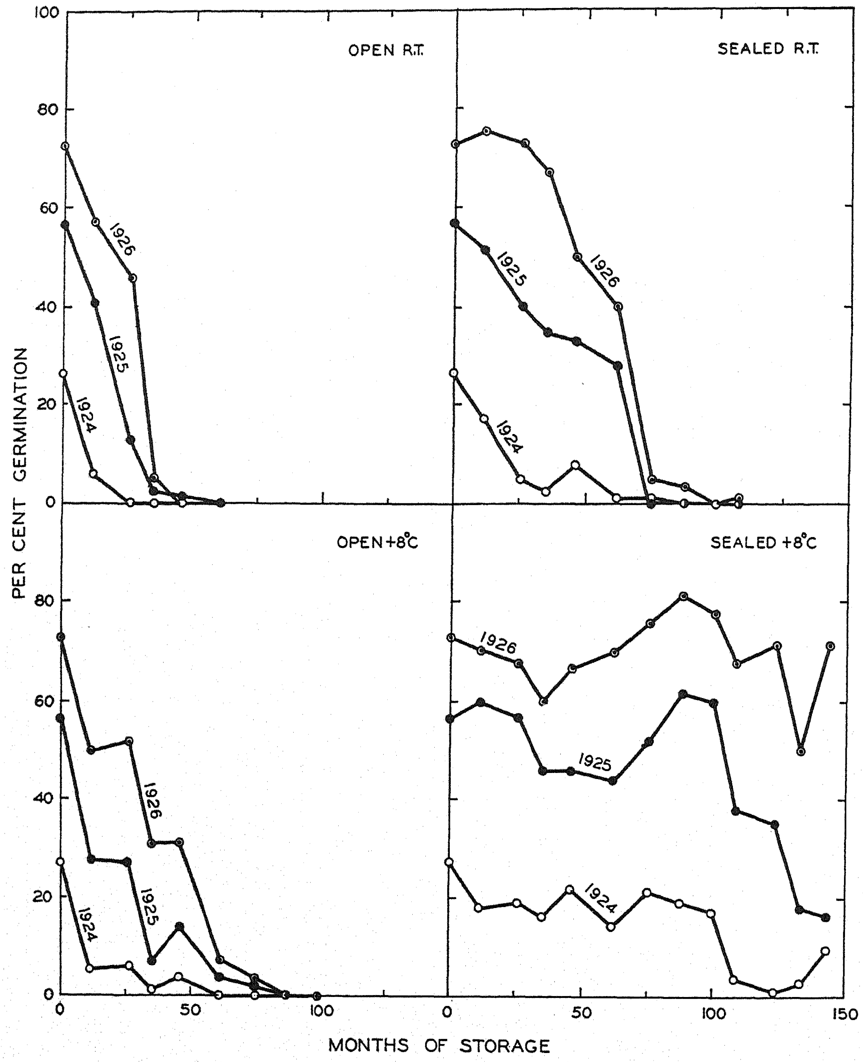

FIGURE 5. Vitality of annual delphinium seeds of the 1924, 1925, and 1926 crops as shown by germination tests on moist filter paper at 15° C. after storage in open or sealed containers at room temperature (R.T.) or +8° C. for different lengths of time after December, 1926.

stored in December, 1926, kept their vitality unimpaired for 143 months.

In addition to testing periods shown in Figures 5 and 6, 69, 81, 93, and 111 months were used. Some of the germination percentages obtained on the basis of duplicate tests of 100 seeds each on moist filter paper at a constant temperature of 15° C. are shown in Table VI. Only those storage conditions which have permitted retention of vitality for some of these periods are shown in the table. Again it will be seen that annual seeds of the 1926 crop apparently retained complete vitality for 143 months in sealed vials at 8° C. The same statement may be made for perennial seeds of the 1926 crop sealed at −15° C. Annual seeds of 1924 and 1925 crops as well as perennial seeds of the 1925 crop have also remained viable under these storage conditions.

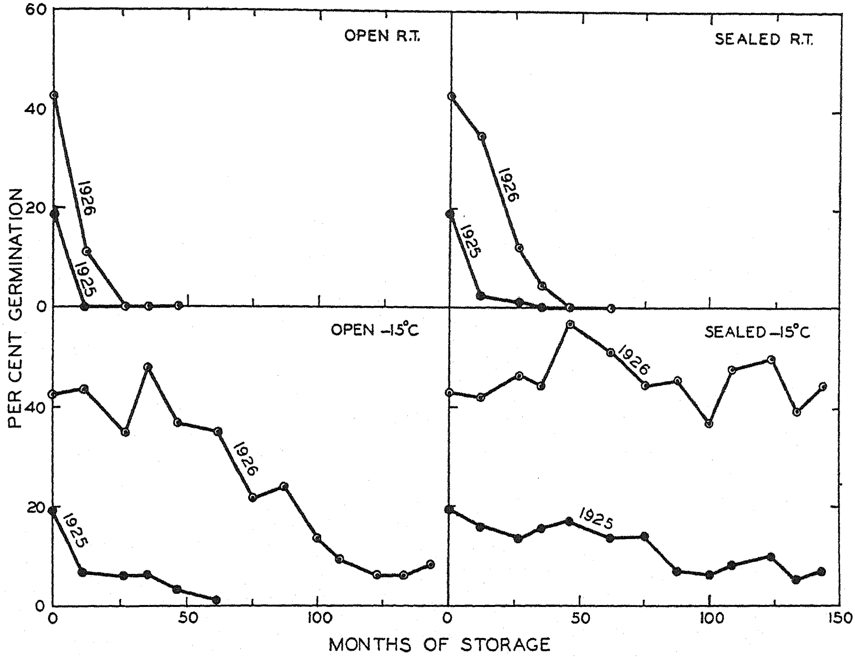

FIGURE 6. Vitality of perennial delphinium seeds of the 1925 and 1926 crops as shown by germination tests on moist filter paper at 15° C. after storage in open or sealed containers at room temperature (R.T.) or −15° C. for different lengths of time after December, 1926.

Deeter (13) obtained 90 per cent germination from delphinium seeds stored in an envelope at room temperature at Danville, Pennsylvania, for five and one-half years. The report of Goss (14) included five lots of delphinium, three of which were good for only the first test (after one year), one failed after the third year, and the remaining one after the fourth year. Apparently then different seed harvests vary somewhat in their

TABLE VI

GERMINATION PERCENTAGES OBTAINED AT 15° C. FROM DELPHINIUM SEEDS STORED UNDER VARIOUS CONDITIONS FOR DIFFERENT LENGTHS OF TIME

Storage	Seed		Germination percentages after storage for months following December, 1926										
	Annual or perennial	Crop	69	75	81	87	93	99	108	111	123	133	143
Sealed 8° C.	Annual	1924 1925 1926	32 60 80	21 52 75	26 57 80	19 61 81	18 56 84	17 59 77	4 37 67	8 44 76	1 35 71	3 18 50	10 16 71
	Perennial	1926	34	28	31	27	21	22	15	16	5	4	2
Sealed −15° C.	Annual	1924 1925 1926	40 60 72	40 68 72	37 59 73	35 66 —	32 67 —	34 59 —	8 37 —	22 55 —	13 48 —	5 39 —	28 59 31
	Perennial	1925 1926	9 44	14 45	16 48	7 46	8 38	6 37	8 48	10 49	10 50	5 39	7 45

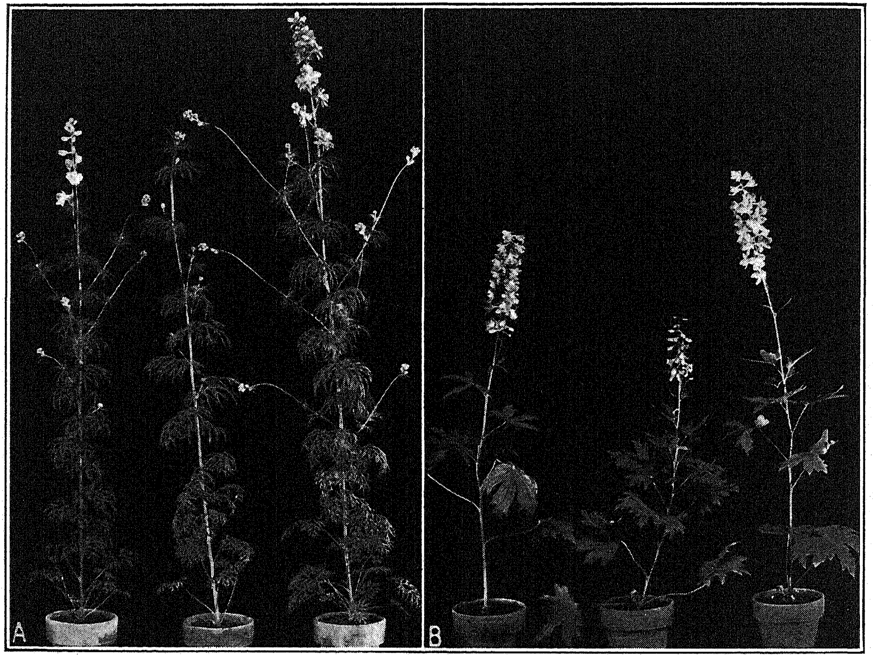

FIGURE 7. Delphinium plants produced from seeds stored for 123 months before planting. (A) Annual and (B) perennial plants.

longevity, but their life-span can undoubtedly be increased by storage under favorable conditions. It is likely that optimum conditions were not used in the present experiment since it has been shown above that better results are obtained when the sealed containers are not opened from the time of storage until the end of the storage period.

Soil tests made after 81, 93, 99, 108, 123, and 143 months of storage have shown that these old seeds were not only capable of germinating on filter paper but also produced seedlings in soil. Some of the seedlings thus produced from seeds taken from the best conditions after 123 months of storage were grown to maturity. The appearances of typical annual and perennial plants are shown in Figure 7.

These seedlings compared favorably at every stage of development with those obtained from fresh seed planted at the same time. Seeds produced on the plants from old seeds were viable and when planted produced normal seedlings.

DISCUSSION

As the data available on best storage methods for many kinds of seeds increase, the uniformity of the response of widely different seed forms to moisture and temperature effects becomes increasingly significant. Low moisture content combined with low temperature represents good storage conditions for most seeds. These two factors are closely interrelated so that the necessity for one depends upon the other. For safe storage of seeds with high moisture contents, low temperatures are required and the higher the moisture content, the lower the required temperature. On the other hand, even at 5° C., which is ordinarily considered sufficiently low for retention of vitality of most seeds, deterioration is rapid if the moisture content of the air is high. Under high humidity conditions, then, temperatures below freezing might be necessary. As the moisture content is reduced, the seeds become able to withstand increasingly high storage temperatures. A limit of course exists. There may be danger of too much drying, especially in coniferous forms (4), and excessive heat is fatal under any conditions. Heinrich (17) found that with free access of air, the viability of many seeds was not prolonged by previous drying but artificial drying was decidedly beneficial when 30° C. was used for storage. For safe storage of Chewings fescue seed over an extended period, the moisture content should not be more than 8 per cent at 30° C., 10 per cent at 20° C., and 12 per cent at 10° C., according to Kearns and Toole (18). Poptzoff (21) working with tobacco seeds found that the higher the storage temperature used, the lower must be the relative humidity. He further claimed that a lower limit of relative humidity did not exist since the lower it is, the greater the certainty of conserving the seed quality. Drying has also been found advantageous for some seeds which were formerly thought to be killed by desiccation. Among these are poplar (20) and elm (7).

In view of these experimental results obtained by various people, the difficulties encountered in keeping seeds viable in hot, humid regions are readily seen. Sugar cane seeds which usually last only a few weeks in the tropics, were kept viable for 7 to 12 months by Darragh (12), who stored them with 9 grams of calcium chloride per 1000 cubic feet of space. Air-dry seeds could not be sealed satisfactorily because the air was too moist. Kondo and Okamura (19) conserved the germination power of rice seeds by air-tight storage with 10 to 20 per cent moisture below 30° C. They pointed out that storage must be begun during the cold dry season. Spencer (24) also related the difficulty of storing seeds in the tropics. He kept soybeans and peanuts viable for ten months by storing at 55° to 60° F. (approximately 12° to 15° C.). San Pedro (22) concluded that dry-warm storage was better than moist-cool storage for keeping vegetable seeds in the Philippines. The effect of different localities on the vitality of soybeans was reported by Toole and Davidson (26). They said that temperature and humidity determined the relative suitability for storage purposes. They found that two lots containing approximately 9 and 7 per cent moisture, respectively, stored under identical conditions, contained the same amount of moisture in approximately one year but the dried lot consistently maintained a higher germination percentage than that which was not dried. This may have been due to the advantage in keeping quality gained during the time the seeds possessed the lower moisture content (see dandelion and aster data above), and points to the importance of the "critical" moisture content. That this "critical" moisture varies with the type of seeds and with the storage temperature is evident. Williams (27) advised removing excess moisture from grass seed as quickly as possible. He defined as "excess" that moisture present in excess of the normal. Normal moisture was found by him to be 14 per cent for rye grass, 13.6 per cent for timothy, and 13 per cent for cocksfoot. Since fluctuation in moisture content, especially when it has reached the "critical" point, is probably of importance in causing deterioration, a study of the moisture contents of various seeds under different humidity and temperature conditions is now being conducted here.

Although sealed storage in general has been reported beneficial, it is obvious that if the moisture content is too high, sealing is detrimental.

A vacuum has been reported to preserve the vitality of several different kinds of seeds. It was found particularly effective for conifers and elms when other storage conditions were unfavorable (4, 7). Guillaumin (15) found in an experiment lasting 12 years that an atmosphere devoid of oxygen and a vacuum were equally efficient in preserving the vitality of soybean seeds. A favorable effect of vacuum was not found in the experiments here reported. This was not surprising in the cases of aster and verbena since vacuum effects usually show up after much longer storage periods but there was an apparent unfavorable effect of vacuum on seeds of dandelion which contained 7.9 per cent moisture. Further data are needed to explain this effect.

The data presented here indicate clearly that the keeping quality of any particular lot of seed does not depend upon the initial vitality as shown by germination tests at the beginning of the storage period, but upon the storage conditions. Under favorable storage conditions, seeds of poor quality maintain their original germination power as long as do seeds of high quality. This may be seen in Figures 5 and 6 where annual and perennial delphinium seed lots of different initial germinating capacity maintain their vitality equally well. This may also be seen from a comparison of aster and verbena seeds which showed original germination percentages of 88 and 45 per cent respectively (Tables II and III). Similar effects have been noted in other instances (3, 4, 7).

If, on the other hand, storage conditions are unfavorable, various seeds will differ somewhat in their longevity, depending upon the susceptibility of the particular seed to deterioration under conditions of high humidity or high temperature or both and the intensity of the unfavorable storage factors. Thus the 1924 crop of annual delphinium seeds actually lost their germination power before those of the 1925 crop and the 1925 crop in turn before those of the 1926 crop when they were stored open or sealed at room temperature, but the trends are similar (Fig. 5).

One should not assume, then, that low quality seeds cannot be stored with safety. It depends entirely upon the storage conditions, and, if these are favorable, certainly short storage periods may be used with success.

To test the germination power of any kind of seed it is necessary to know the germination requirements. With the exception of annual delphinium and pansy seeds, both of which gave very poor germination if planted at temperatures above 20° C., the seeds of the species reported here presented no special germination problems. The importance of using favorable germination methods in making vitality tests of stored seeds is obvious. If old annual delphinium seeds were planted in a high-temperature greenhouse, for example, the failure to produce seedlings might be attributed to the age of the seed when, as a matter of fact, it could equally well be due to the inhibitive germination temperature. A true test of these seeds requires a temperature of about 15° C. or, if higher temperatures must be used, the seeds should be pretreated on a moist surface for one, two, or three weeks at temperatures of about 15° C., 10° C., or in a refrigerator before planting (5). It will be recalled that Harrold (16) thought some of the germination failures of pansy seeds might be assigned to high soil temperatures. Evidence has been reported in previous papers (4, 7) indicating the importance of proper testing methods for measuring vitality in elm and coniferous seeds.

Reports of ageing effects on seeds should certainly be accompanied by a description of the germination methods employed. The medium as well as the temperature is very important. Both soil and filter paper tests should be used. Germination counts taken as soon as the radicle emerges from the seed are often higher than seedling production counts taken after the shoot appears above ground in soil plantings. Embryos might possess energy enough to be counted as viable in the former case but would not be able to push above the soil. This distinction becomes increasingly important as the seeds decline in viability. It is also important to make frequent observations on the germination conditions so that any variation which might affect the seedlings is noted. For example, the low seedling production values obtained in the greenhouse from dandelion seeds stored two years (Table I) may be explained by the presence of ants which did much damage to the seeds overnight. Again the seedlings of aster and verbena produced from seeds planted after one year of storage were badly infected with damping-off fungus, a condition which did not exist in any of the other soil plantings. Consequently seedlings were lost and the counts were comparatively low (Tables II and III).

It has been found here that plants grown to maturity from old seeds which had been stored under favorable conditions were apparently normal. Not only was this found for both annual and perennial delphinium seed stored for longer than ten years (Fig. 7) but a similar effect has also been noted for carrot, eggplant, onion, tomato, and lettuce plants grown from six-year-old seeds (6). However, an initial retarding effect on development has been shown for tomato and eggplant seedlings (6) produced from seeds stored under unfavorable conditions so that the germination power had been significantly reduced. At later stages, however, these differences in growth disappeared.

Schjelderup-Ebbe (23) also found that plants from old seeds grew and flowered normally and produced normal seeds which in turn produced a second generation of normal plants.

Crocioni (10), on the other hand, found that the young plants from old seeds developed slowly and had less resistance to adverse conditions. Paralleling the age of the seed, the seedlings were smaller throughout in wheat and *Brassica*. In legumes, however, this effect was evident only in very old seeds.

Chromosome disturbances reported in plants from old seeds have been in those from unfavorable or at least not optimum storage conditions. Although Stubbe (25) found that the gene mutation rate in *Antirrhinum* increased with the age of the seed, he stated that the amount of mutability was not directly related to age. This variation he thought might be due to external conditions or to molecular changes within the embryo. Here again there was an indication that storage conditions and not the age of the seeds determined whether plants were normal or abnormal.

It is to be expected that with sufficient age the embryo in the seed will become weakened and will produce inferior or abnormal plants. This age doubtless varies with the individual species and depends largely on the storage conditions. It is safe to assume, however, that under favorable conditions of storage seeds not only have their period of viability greatly extended but also maintain the ability to produce normal seedlings for long periods of time, usually for periods far in excess of any that would normally be used in the seed trade.

No attempt has been made here to cite all of the published work on longevity of seeds, but rather papers have been selected to illustrate certain salient points. A complete review of this subject has been written by Crocker (11).

SUMMARY

Data are given for some flower seeds showing retention of vitality after storage for various periods up to 12 years.

Dandelion (*Taraxacum*) seeds could be stored safely at room temperature for three years if the moisture content was reduced to 5 per cent or less and if the containers were not opened until the end of the period. If the containers were opened as often as every six months it was necessary to reduce the moisture content to as low as 3.9 per cent at the beginning of the storage period in order to keep the seeds viable for three years. Sealed storage proved superior to open storage regardless of moisture content (up to 7.9 per cent) when room temperature or 5° C. was used. Seeds kept perfectly at $-5°$ C. for the three-year period regardless of sealing or moisture content.

Similar trends in behavior were found for seeds of aster (*Callistephus*) and *Verbena* stored in the same manner as those of dandelion. In all three of these forms there were indications that fluctuation in moisture content was deleterious after the seeds had absorbed water up to the "critical" point. This was shown in data in which seeds with various moisture contents placed in hermetically sealed tubes not opened from the time of storage until the time of testing were compared with those in containers which were opened and re-sealed at intervals.

Sweet pea (*Lathyrus*), pansy (*Viola*), and *Venidium* seeds remained viable for approximately three years under favorable storage conditions.

Seeds of the regal lily (*Lilium regale*) were kept fully viable for at least six years under conditions of dry, cold storage. If the atmosphere of the storage chamber was high in humidity, it was necessary to place air-dry seeds (containing not more than 10 per cent moisture) in sealed containers, unless the temperature was below freezing. These seeds were also kept viable at room temperature by reducing the moisture content and placing in sealed containers.

Air-dry seeds of annual and perennial *Delphinium* retained their original germination power unimpaired for 143 months of sealed storage at 8° C. and −15° C., respectively. Furthermore, normal seedlings were produced from these old seeds.

Results of the experiments reported here indicated that the keeping quality of seeds depended upon the storage conditions and not upon the original vitality. Seeds of poor quality remained viable as long as those of good quality under favorable conditions of storage.

In all cases combination of high moisture content and a temperature of 5° C. brought about deterioration at approximately the same rate as lower moisture content and room temperature. A temperature below freezing was necessary to keep seeds viable under high humidity conditions. This should be kept in mind when commercial seed lots are placed in cold storage rooms.

A discussion of some of the factors involved in retention of vitality is included.

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LIFE OF GLADIOLUS POLLEN PROLONGED BY CONTROLLED CONDITIONS OF STORAGE

Norma E. Pfeiffer

It was previously found possible by means of controlled conditions of temperature and humidity to extend the life duration of pollen of *Lilium* species and of *Amaryllis*, far beyond that under usual room conditions (4, 5). Some of the methods for storing pollen which had proved successful for these two ornamentals were tried for a number of varieties of *Gladiolus*. Although there is much greater variation in results than with lily species, it is found possible to prolong the life of the pollen to a matter of weeks instead of days. The results are of interest especially to those endeavoring to cross *Gladiolus* species or varieties which cannot be brought into bloom at the same time or in the same place.

METHODS

Storage. Samples of pollen were collected usually by removing the stamens just before dehiscence, drying them slightly in clean open dishes, and then storing either in gelatin capsules or in open vials in desiccators. The humidity in the desiccators was controlled by means of saturated solutions of salts; calcium chloride, magnesium chloride, and potassium carbonate were selected, giving a range from 35 per cent relative humidity to more than 44 per cent at 10° C. In addition, there was one desiccator with a solution of sulphuric acid to give a humidity of 50 per cent. The desiccators were kept in the dark, at 10° C. When pollen was taken out, the container was returned to storage as quickly as possible.

Criterion for viability. In the earlier work it was possible to test the viability of the pollen by germination on an artificial medium, a method giving adequately uniform results to be satisfactory. Pollination with the stored pollen and observation of the capsule and seed production resulting was a better but more time-consuming method, requiring a second crop of flowers. After long intervals of storage, lily pollen sometimes germinated on the stigma when samples from the same lot no longer germinated on the artificial medium.

In the winter of 1937–1938, tests were made with artificial media in an endeavor to find a successful one for *Gladiolus*, at the same time that preliminary experiments on storage were carried on. The pollens available were those of hybrid forms derived from native South African species by Forman T. McLean and were nearer to the species than are most garden varieties. These pollens gave fairly uniform results when sown on a medium containing 10 per cent cane sugar, 1 per cent agar, and as recommended by Brink (1) the addition of yeast. The preliminary storage tests showed that for the limited number of forms tried, the humidities resulting from the use of saturated solutions of magnesium chloride, calcium chloride, and potassium carbonate were more favorable than a higher one provided by magnesium nitrate or a 65 per cent humidity maintained by a solution of sulphuric acid; these, however, also increased the longevity of the pollen to some extent. Examples may be cited for the effectiveness of the magnesium chloride desiccator, where 19 and 12 per cent germination were obtained in duplicate tests after 59 days' storage of one sample; of potassium carbonate, 19 and 19 per cent germination after the same interval. At the same time, pollen over magnesium nitrate or in 65 per cent humidity gave a fraction of a per cent germination.

A few garden varieties were tried with the same medium during this winter, with variable results. This work was extended in the summer of 1938, when many varieties were available in the field. This medium was found to give low as well as less consistent results in paired mounts of the same variety than the forms used earlier. Modifications of the sugar content and agar concentration, addition of stigma or crushed style tissue or orange juice, and control of the temperature failed to give positive results with some varieties and consistent positive results with others. The viability was then tested by the more tedious method of controlled pollinations. These were made on a limited number of varieties brought into bloom in the greenhouse.

Pollinations. Stigmas of flowers which had previously been emasculated were pollinated with the stored pollen. It was unnecessary to bag the flowers since only a limited number were in bloom at one time and these were regularly emasculated. Although some of the varieties used, like Purple Glory, were undoubtedly self-sterile, similar precautions of removing stamens early were taken with all. The number of pollinations per test ran from one to six, depending upon the number of flowers and the amount of pollen available.

Even with this method, not all pollinations were effective in determining the viability of the pollen. Unanticipated incompatibility or self-sterility, where it was evident that the seed parent also played a decisive rôle, sometimes blocked positive results. Some apparently good capsules were set which later proved to be filled with phenospermic seeds. Some varieties showed a high proportion of sterile pollen grains at the start, which would result in a low seed set after storage, no matter how effective the storage method. It was further observed that under winter conditions in a greenhouse, the same variety from different sources grown under different conditions showed very different proportions of fertile pollen. Usually White Butterfly has a large number of well filled grains which germinate readily. However, one lot was found to have many undeveloped grains in the pollen sample, of which no grains showed ability to germinate on the same medium. Microscopic observation of the relative number of good and poorly developed spores gives some clue as to the original possibilities of the pollen to be used and stored.

Germination of resulting seed. In all the earlier tests, seed resulting from pollinations with stored pollen was counted, planted in flats, and germinated in soil in a cool greenhouse. Originally counts of seedlings were made after a month, but when it was found that additional strong seedlings continued to appear with a longer interval, counting was deferred to six or eight weeks after planting. This later counting equalized the unevenness of sprouting that occurred in lots of seed from different combinations of parents as well as allowed for the effect of the low light intensity in December and January.

RESULTS

Viability of Gladiolus pollen. Under ordinary conditions of temperature and moisture, the life duration of *Gladiolus* pollen is much shorter than that of *Lilium*. Growers indicate that the effectiveness of the pollen is greatly reduced by the end of twenty-four hours after shedding and is practically nil in two days. Pollinations designed to determine the longevity of the pollen were made in the greenhouse in winter. It was found that even fresh pollen gave a variable seed set; with no drying the set was frequently low. In some combinations of parents, there was no seed set at any age of the pollen, indicating incompatibility or self-sterility. In most varieties the pollen failed to give good results after two days, but Souvenir and White Butterfly were two exceptions which still gave seed after four days under room conditions. These two varieties also proved especially resistant to long storage under controlled conditions.

Viability of stored pollen. The following varieties were used either as pollen or seed parent or both: Alice Tiplady, Picardy, Purple Glory, Salmon Star, Senorita, Souvenir, White Butterfly; in lesser degree, Flaming Sword, Halley, Queen of Bremen, Scarlet Princeps, and Zona. These bloomed in the greenhouse, not all at one time, starting in October; pollinations were made from that time to the last of February. Intervals of storage of pollen ranged by uneven steps from a few days to over a hundred days. The pollen stored for the shorter intervals of time came from the winter-grown stock; that stored for the very long intervals was collected from flowers blooming in the field the previous August. Under these conditions, some varieties, like Alice Tiplady and Queen of Bremen, seemed to be poor seed parents, while Purple Glory, very unsatisfactory for pollen, gave relatively good sets of seed with pollen from other varieties and strong seedlings from this seed.

In general, all four humidities in the desiccators in any series, at a temperature of 10° C., were advantageous in prolonging the life of the pollen stored. Of the four, the humidity resulting from the use of a saturated solution of potassium carbonate usually gave the best results.

TABLE I

AVERAGE SEED SET RESULTING FROM POLLINATION WITH POLLEN STORED AT 10° C. IN CONTROLLED HUMIDITIES

Parents		Storage interval, days	Average seed set over			
Seed	Pollen		Magnesium chloride	Calcium chloride	Potassium carbonate	Sulphuric acid 50% humidity
Purple Glory	Senorita	30–34	20	24	41	25
		40	11	—	—	—
		42	—	—	41	—
		50	—	11	—	—
		114	—	—	—	1/4
Salmon Star	Senorita	26–29	23	23	26	—
		114	—	—	—	5
Purple Glory	Salmon Star	29	—	41		
		23	9	—		
		67	—	22		
		60	1	—		
Salmon Star	Salmon Star	36	10	—		
		38	—	17		

As seen in Table I, Senorita pollen on Purple Glory stigmas (Fig. 2 B 2, 3, 4, 5, 6) and on Salmon Star stigmas gave the highest average number of seeds per capsule after storage over potassium carbonate, when the storage interval was about a month.

The most advantageous storage condition may be judged not only by a better seed set after any given interval of storage, but also by the ability to set seed well after a long period. In Table I results of pollinations on Purple Glory with Senorita pollen stored 40 to 50 days show a continued high average number of seeds following storage over potassium carbonate. The supply of pollen of this lot ran out at this time, except in the desiccator at 50 per cent; with this last pollen only one seed was set in four pollinations on Purple Glory and an average of 5 seeds in three pollinations on Salmon Star, after 114 days' storage.

From a less complete series of pollinations with Salmon Star pollen on Purple Glory and Salmon Star (Table I), storage over calcium chloride proved more advantageous than over magnesium chloride. Capsules resulting from pollinations with pollen stored for different intervals are shown in Figure 1.

Most pollen was found to give adequate results under the most effective storage conditions for a period in the neighborhood of six to eight weeks. Thereafter the drop in seed production indicated a marked decrease in viability; for most varieties only rarely were seeds set to any extent if the storage interval exceeded nine or ten weeks. Two varieties proved exceptional in this regard; White Butterfly and Souvenir were remarkable for the long intervals over which their pollen retained ability to function. Data in Table II show the seed set with these pollens on different varieties after longer intervals of storage, and the capsules are shown in Figure 1 A, 1 C 10, 11, 14, 15, 18, 20, 21.

TABLE II

AVERAGE SEED SET RESULTING FROM POLLINATION WITH POLLEN STORED AT 10° C. FOR LONGER INTERVALS

Parents		Storage interval, days	Average seed set	Desiccator containing
Seed	Pollen			
Purple Glory	Souvenir	86	13	Sulphuric acid*
Senorita	Souvenir	81	26	Magnesium chloride
Salmon Star	Souvenir	86	19	Sulphuric acid*
" "	"	94	11	" "
Purple Glory	White Butterfly	102	30	Sulphuric acid*
Senorita	White Butterfly	89	30	Magnesium chloride
"	" "	83	34	Calcium chloride
Salmon Star	White Butterfly	103	30	Sulphuric acid*

* Relative humidity of 50%.

Seedling production. The ability of the seed to germinate and produce strong seedlings was tested in the earlier crops in addition to the making of seed counts. Of 72 seed lots, with various combinations of seed and pollen parents and with various storage intervals and humidities, about half (35) gave a germination of 75 per cent or better. All but seven gave at least 50 per cent germination, and five ranged between 90 and 100 per cent germination. Some of the seedling stands are illustrated in Figure 2. In several lots, some seedlings lacking in chlorophyll content to a greater or less degree grew vigorously for a time, but succumbed eventually through the failure of a food supply.

Restoration of germinating capacity of pollen. As indicated above, the viability of pollen stored at room temperature and humidity appeared to be of short duration, usually with inability to produce seed in controlled pollinations after a few days. A suggestion that such pollen was not necessarily dead and that its germinating capacity might be restored through subsequent storage at a higher humidity and a lower temperature came from the work of Nebel and Ruttle (3). Using apple pollen which had apparently lost its vitality after storage in the laboratory for five weeks,

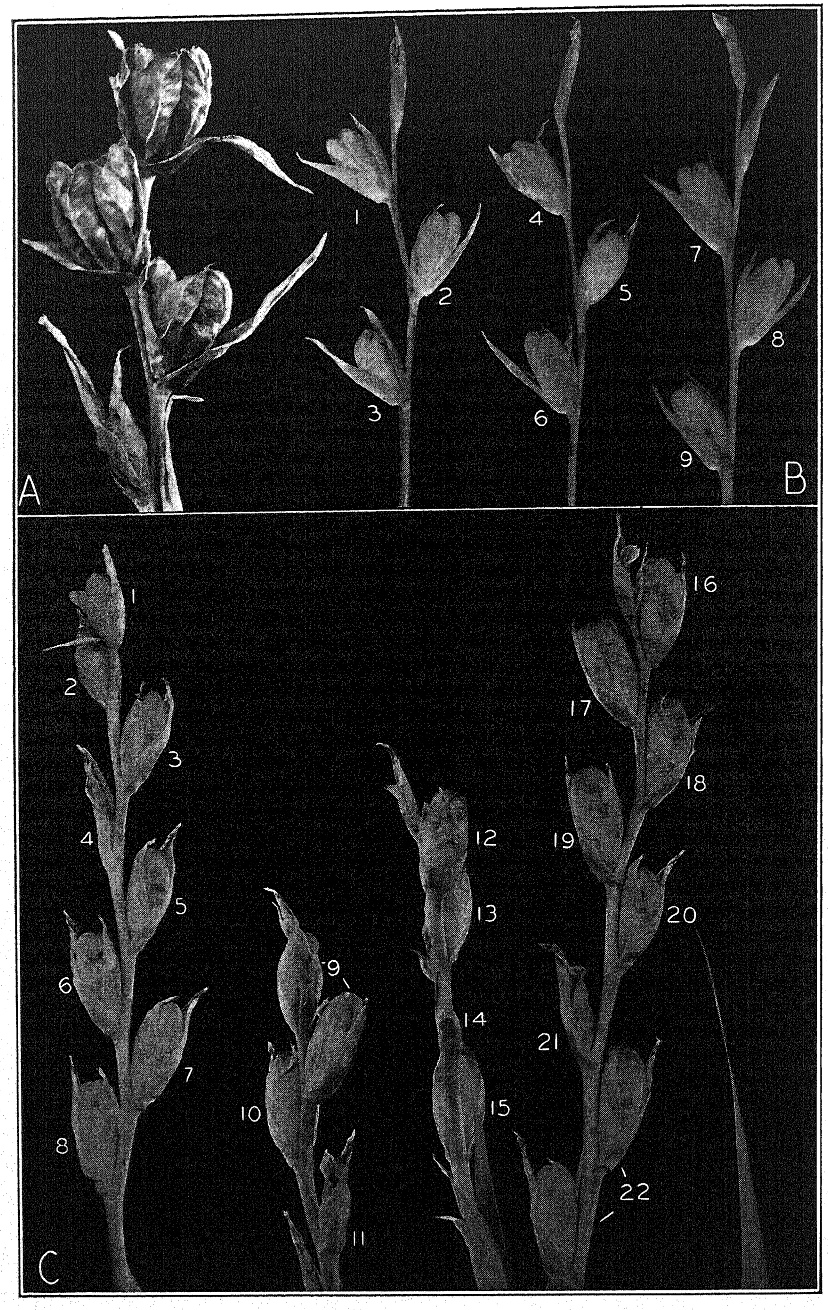

FIGURE 1. (For description see legend on opposite page.)

they "revived" it by placing it in storage at 2° to 8° C. at 80 per cent humidity.

Several different collections of White Butterfly, of Souvenir, and of Picardy pollen were stored at first at room temperature and humidity, followed by storage in a desiccator providing 65 per cent humidity, at 10° C. Pollinations were made at the end of the interval under room conditions, and again at short intervals after storage at the low temperature at a higher humidity. There was a clear tendency toward an increase in ability of the pollen to germinate and function in setting seed after placing it under controlled conditions. One lot of White Butterfly pollen on White Butterfly stigmas gave a total set of 16 seeds in four pollinations after six days under room conditions; after the next four days at 10° C. and 65 per cent humidity, there was an average set of 36 seeds in six pollinations; after 7, 10, and 13 days in the desiccator when the total storage intervals were 13, 16, and 19 days, the average seed set was 4, 5, and 6 seeds, approximately that at the end of the first four days. In another sample kept under room conditions for eight days, the successive pollinations at three-day intervals after placing in the desiccator gave average sets of 16, 15, 21, and 3 seeds (total storage intervals of 11, 14, 17, and 20 days respectively).

Used on Queen of Bremen stigmas, White Butterfly pollen stored under room conditions six days followed by seven and thirteen days in the desiccator gave 17 seeds per capsule, in excess of ten seeds produced by very fresh pollen. On the same seed parent, Souvenir pollen stored for three days under room conditions followed by nine and twelve days in the desiccator gave average seed sets of 9 and 5 seeds; another lot stored six days under room conditions followed by six, nine, and twelve days in the desiccator, gave average sets of 10, 8, and 15 seeds. Also on Queen of Bremen, Picardy pollen which when fresh gave the low set of six seeds in a

FIGURE 1. *Gladiolus* capsules from pollination with pollen stored at 10° C. (A) Senorita ♀ × White Butterfly ♂, 83 days at 50% humidity. (B) White Butterfly ♀ × Picardy ♂, except 8. 1. Pollen 56 days over magnesium chloride. 2, 3. Air-dry 4 days. 4. Air-dry 1 day, followed by 6 days in 65% humidity. 5. Over potassium carbonate 54 days. 6. Over calcium chloride 53 days. 7. Air-dry 4 days followed by 6 days in 65% humidity. 8. Souvenir ♂, air-dry 6 days followed by 5 days in 65% humidity. 9. Picardy ♂, 6 days over potassium carbonate. (C) Purple Glory ♀. 1. × Salmon Star, 28 days over calcium chloride. 2. × Senorita, 39 days over potassium carbonate. 3, 4. × Salmon Star in 50% humidity 22 and 112 days respectively. 5–9. × Senorita. 5. Over calcium chloride 33 days. 6. Over potassium carbonate 21 days. 7, 8. Over magnesium chloride 31 and 27 days. 9. Over potassium carbonate 33 days. 10. × White Butterfly in 50% humidity 102 days. 11, 14, 20, 21. × Souvenir at 50% humidity 94 days (poor capsules, low seed set). 12, 16, 17. × Salmon Star over calcium chloride 16 days. 13. × Senorita over potassium carbonate 29 days. 15, 18. × White Butterfly in 50% humidity 94 days. 19. × Senorita in 50% humidity 13 days. 22. × Senorita over calcium chloride 9 days.

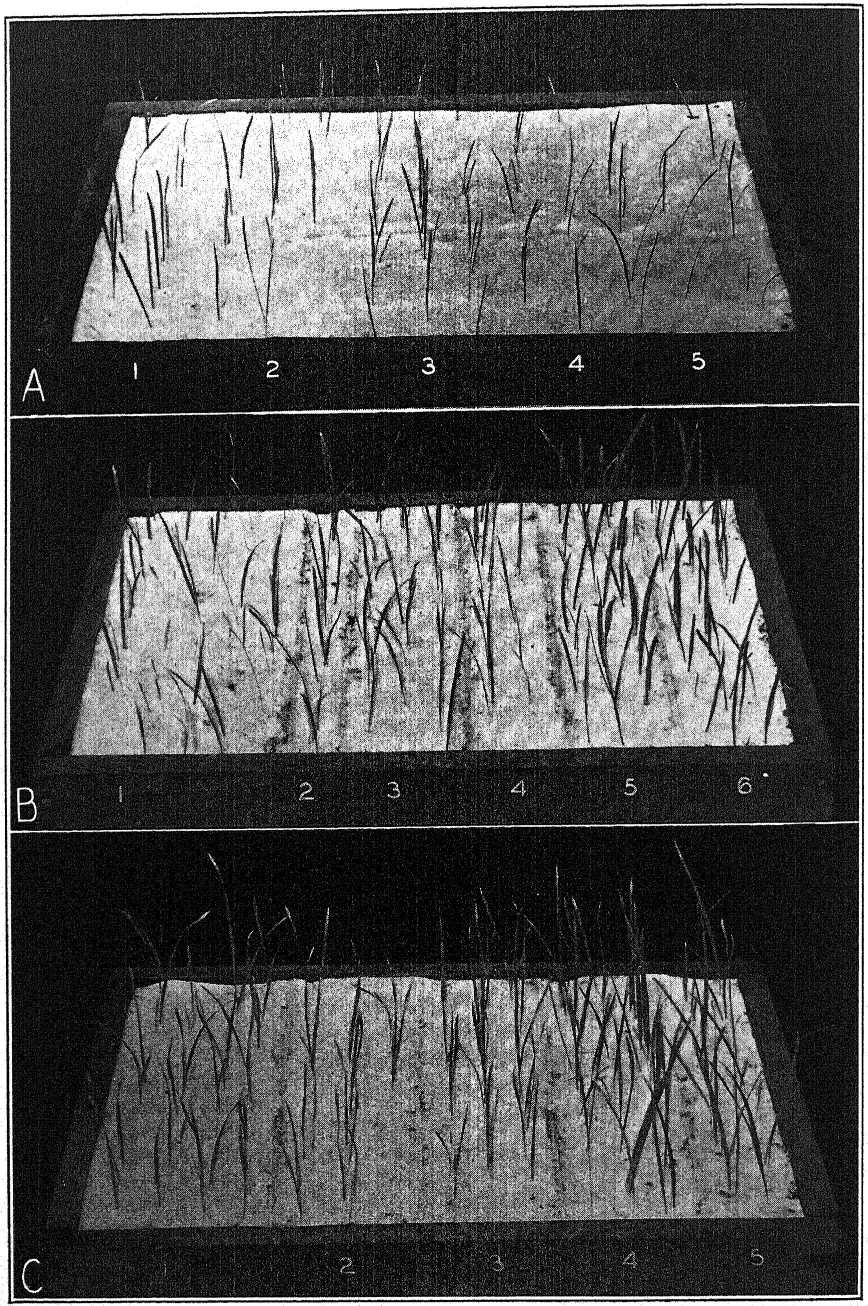

FIGURE 2. (For description see legend on opposite page.)

capsule, produced an average of seven seeds after 15 days in the desiccator subsequent to two days under room conditions, and an average of five seeds after 12 days in the desiccator subsequent to four days under room conditions.

Picardy pollen after one day under room conditions gave 16 seeds per capsule with White Butterfly as seed parent; subsequent to additional storage intervals of 3, 6, 9, 12, and 15 days in the desiccator at 10° C., the average set was 28, 28, 26, 32, and 15 seeds per capsule. Another lot held two days under room conditions gave 12 seeds in two pollinations; used for pollinations after storage in the desiccator for 3, 6, 9, 12, and 15 days, the same pollen resulted in seed numbers of 19, 13, 20, 20, and 15 per capsule respectively. A third lot of pollen held four days under room conditions resulted in 12 seeds per capsule; under desiccator conditions 3, 6, 9, and 12 days later, the set ran 29, 33, 19, and 23 seeds per capsule respectively.

Souvenir pollen used on Souvenir stigmas gave a set of 52 seeds when fresh; after 2, 3, and 6 days at room temperature, the set was 30, 33, and 26 seeds respectively. Removed to the desiccator for nine days, that previously held for six days under room conditions gave 58 seeds per capsule. Similar results occurred with the use of Souvenir pollen on White Butterfly, where seed sets of 33 and 32 resulted after three and six days under room conditions. The three-day-old sample placed in the desiccator 3, 6, and 9 days, gave averages of 50, 38, and 28 seeds per capsule respectively. The six-day-old sample after similar storage intervals in the desiccator gave 37, 40, and 35 seeds per capsule respectively.

Results of pollinations after such storage of pollen of White Butterfly, Souvenir, and Picardy, on stigmas of four varieties are shown in Table III. The seed set after the pollen is held in 65 per cent humidity at 10° C. is greater (column 6) than that with earlier pollinations immediately after storage under room conditions (column 5). Capsules resulting from some pollinations are illustrated in Figure 1 B 2, 3, 4, 7, 8.

Scattered tests with samples of other varieties also indicate an increase in germinating ability above that shown by pollen stored under room conditions, brought about by subsequent storage under these particular con-

FIGURE 2. *Gladiolus* seedlings, from seed produced by pollination with pollen stored at 10° C. (A) Senorita ♀. 1, 2, 3. × White Butterfly, 83 days in 50% humidity. 4, 5. × Senorita, 6 and 7 days over potassium carbonate. (B) 1. Salmon Star ♀ × Senorita, 38 days over potassium carbonate. 2, 3, 4, 5, 6. Purple Glory ♀ × Senorita, respectively 50 days over calcium chloride, 49 days over magnesium chloride, 20 days over calcium chloride, and 42 days over potassium carbonate. (C) Salmon Star ♀. 1. × White Butterfly, 103 days in 50% humidity. 2. × Senorita, 30 days over magnesium chloride. 3. × Souvenir, 94 days in 50% humidity. 4. × Senorita, 24 days over calcium chloride. 5. × Salmon Star, 54 days over magnesium chloride.

ditions of humidity and temperature. No test of humidities other than 65 per cent was made, so that it is not known whether this is the most favorable for resumption of the capacity to function, and no test of the effect of temperatures other than 10° C. was made. This combination, however, appears to be advantageous in making good use of pollen which for any reason has been exposed to drying. The longest interval after which re-

TABLE III

AVERAGE SEED SET AFTER STORAGE UNDER ROOM CONDITIONS, FOLLOWED BY STORAGE AT 10° C. AND 65 PER CENT HUMIDITY

Parents		Fresh	Storage						
Seed	Pollen	Seeds		Room	10° C. and 65% humidity				
White Butterfly	White Butterfly		Days	6	4	7	10	13	
			Seeds	4	36	4	5	6	
			Days	8	3	6	9	12	
			Seeds	—	16	15	21	3	
Queen of Bremen	White Butterfly	10	Days	6	7	13			
			Seeds	—	17	17			
Queen of Bremen	Souvenir		Days	3	6	9	12		
			Seeds	—	—	9	5		
			Days	6	6	9	12		
			Seeds	—	10	8	15		
Souvenir	Souvenir	52	Days	2					
			Seeds	30					
			Days	3					
			Seeds	33					
			Days	6	9				
			Seeds	26	58				
White Butterfly	Souvenir		Days	3	3	6	9		
			Seeds	33	50	38	28		
			Days	6	3	6	9		
			Seeds	32	37	40	35		
Queen of Bremen	Picardy	6	Days	2	15				
			Seeds	—	7				
			Days	4	12				
			Seeds	—	5				
White Butterfly	Picardy		Days	1	3	6	9	12	15
			Seeds	16	28	28	26	32	15
			Days	2	3	6	9	12	15
			Seeds	6	19	13	20	20	15
			Days	4	3	6	9	12	—
			Seeds	12	29	33	19	23	—

covery is possible has not been determined, but it is apparent from the tests that several days under room conditions still allow for recovery.

DISCUSSION

Few reports have appeared on storage of pollen of Iridaceae under controlled conditions. Tests have been reported for only one genus, in this case using germination on an artificial medium. *Iris graminea* and *I. pseudacorus*, according to Pfundt (6) who compared longevity under air-dry conditions, 30, 60, and 90 per cent relative humidity, and over concentrated sulphuric acid, at 17.5° to 20° C., gave best results under the driest conditions. *I. graminea* pollen, surviving 20 days under air-dry conditions, lived but three days at 90 per cent humidity, 16 days at 60 per cent, and 48 and 57 days at 30 per cent humidity and over sulphuric acid respectively. *I. pseudacorus*, remaining alive 12 days when air-dry, survived 29 days at 30 per cent humidity and over sulphuric acid. He further recorded data on pollen of other members of the Liliales, five of which (*Luzula angustifolia*, *Colchicum autumnale*, *Tulipa gesneriana*, *Galanthus nivalis*, *Leucojum vernum*) gave best results in 30 per cent humidity, and next best over sulphuric acid, while two (*Lilium bulbiferum*, *Agave densiflora*) were equally successfully stored under these two conditions and two others (*Hemerocallis fulva*, *Aloe longearistata*) survived somewhat better in the very dry atmosphere over sulphuric acid than at 30 per cent humidity. It appears that the larger number were stored to advantage at 30 per cent humidity. The advance to the next higher humidity used, 60 per cent, is great enough to overstep those especially used in the present work. The same thing is true of the work of Holman and Brubaker (2) who tested germination in humidities of approximately 27, 63, and 92 per cent, as well as air-dry and very dry, 0.005 per cent humidity, at 17° to 22° C. The three forms of Liliales (*Smilacina amplexicaulis*, *Trillium sessile*, *Zygadenus fremontii*) for which these authors give data were all favored more by storage at 27.2 per cent humidity than by either higher humidities or air-dry conditions.

More recently it has been reported that *Iris* pollen (7) can be used successfully for pollinations after one to three weeks' storage of the whole stamen upon absorbent cotton lying on a small piece of calcium chloride in a glass vial, kept at 40° F. when not in use. Here advantage is taken of a lower temperature, comparable to that used in the present work, although the moisture content is less definite than those obtained in the desiccators.

SUMMARY

Viability of pollen of *Gladiolus* was tested by means of pollinations and capsule and seed production when germination on artificial media gave too variable results for comparison.

The life of *Gladiolus* pollen was prolonged to eight or even ten weeks.

Pollen of a few varieties produced some seed even after 102 days' storage.

The most successful method of preserving viability was storage in the dark at 10° C. in a humidity controlled by a saturated solution of potassium carbonate or in 50 per cent humidity, controlled by a sulphuric acid solution.

Good results, although with slightly lower seed sets, were also obtained from storage over saturated solutions of magnesium chloride and calcium chloride at 10° C.

Most varieties show greatly reduced vitality within two days if stored under room conditions of temperature and humidity.

Exceptional length of life is characteristic of a smaller number, such as White Butterfly and Souvenir.

Pollen kept under room conditions for two, four, or even six days, depending upon the variety, may recover its ability to function when placed in an atmosphere with 65 per cent humidity at 10° C., as indicated by seed set by means of pollen stored thus for 3, 6, 9, or 12 days.

Seed produced by pollination with pollen stored for various intervals from 10 to 102 days gave a good stand of strong seedlings; about half of the seed lots tested, with various combinations of parents, gave a germination of 65 per cent or higher.

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A METHOD FOR THE QUANTITATIVE DETERMINATION OF GLUCOSE AND FRUCTOSE IN THE PRESENCE OF PENTOSES[1]

Forrest E. Haver, Jr. and Jack Compton

An investigation of reducing sugars in the cytoplasm of developing cotton fibers led to the evolution of a procedure which would provide a rapid means for determining quantitatively the amounts of glucose and fructose in the presence of pentoses.

The ferricyanide method, originated by Hagedorn and Jensen (2, 3) as modified by Hanes (4) for determining total reducing sugars, was found applicable over the wide range of sugar concentrations encountered. The formula given by Hulme and Narain (6) for converting titration into milligrams of reducing sugar was used in the calculations in preference to reading the result from a graph.

The Willstätter iodimetric procedure (1, 12) for aldose determination was used to estimate the amount of glucose and pentoses in the various mixtures. The effect of fructose, a ketose sugar, on the aldose determination was also investigated. As shown in Table II, a correction of 4 per cent adequately discounts the error due to the simultaneous reducing action of fructose, and in most cases need not be considered at all.

The MacLeod and Robison (7) adaptation of the Willstätter iodimetric procedure for the estimation of aldoses on a micro-scale was successfully employed when the amount of sample was limited.

The removal of hexoses by selective yeast fermentation from hexose-pentose mixtures was suggested by the observations of Somogyi (9, 10), Van Slyke and Hawkins (11), and by Raymond and Blanco (8). The present investigation corroborates the findings of these workers showing that the selective yeast fermentation is conditioned by the concentration and composition of the sugar mixtures. Proper control of these variables was found essential in obtaining reliable quantitative results.

The combining of these methods, with slight modifications, has led to a procedure for determining quantitatively the amounts of glucose and fructose present in mixtures with pentoses.

EXPERIMENTAL

APPARATUS

Ten cubic centimeter burettes, calibrated to 0.02 cc., were used throughout the experiments. A boiling water bath was used for heating

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the various samples at 100° C. Fresh tap water, 12°–15° C. was used for cooling.

Where filtration of the solutions was necessary, 55 mm. Hirsch filter funnels, equipped with filter paper and Celite, were used. The filtrates were caught in 22×155 mm. test tubes with sealed-in side arms, to which vacuum could be applied to increase the rate of filtration.

MATERIALS

The specific rotations of the various sugars used in this work at equilibrium in water were: d-glucose $[\alpha]_D^{23} = 52.5°$, d-fructose $[\alpha]_D^{23} = -92.5°$, l-arabinose $[\alpha]_D^{23} = 110°$, and d-xylose $[\alpha]_D^{23} = 19°$. The close agreement of these values with those given in the literature (5) for these sugars was taken as proof of their purity.

The reagents used in obtaining the various titrations recorded in the data presented were as follows:

1. The Hanes (4) modification of the Hagedorn and Jensen method for total reducing sugars;

Solution A

8.25 g. of potassium ferricyanide
10.60 g. of anhydrous sodium carbonate

The salts were dissolved in one liter of distilled water and kept in an opaque bottle for two or three days before using.

Solution B

25.0 g. of potassium iodide
50.0 g. of zinc sulfate
250.0 g. of sodium chloride

The salts were dissolved in one liter of distilled water and filtered through two thicknesses of filter paper before using, to remove free iodine.

Solution C

Fifty cubic centimeters of glacial acetic acid diluted to one liter with distilled water.

Solution D

One gram of starch was stirred in 20 cc. cold water and then washed into 60 cc. of boiling water. This was boiled for two minutes after which 20 g. of sodium chloride were added and the solution allowed to cool. It was then diluted to 100 cc. and was ready for use as an indicator.

Solution E

Approximately N/75 sodium thiosulphate

This solution was accurately standardized each day before use.

2. The Willstätter (1) iodimetric procedure;
   - 0.1 N iodine in potassium iodide
   - 0.1 N sodium hydroxide
   - 10 per cent sulphuric acid
   - 0.1 N sodium thiosulphate
3. The MacLeod and Robison (7) modification of the Willstätter iodimetric procedure;
   - 0.02 N iodine in potassium iodide
   - 5 per cent sodium carbonate
   - 0.5 N sulphuric acid
   - 0.005 N sodium thiosulphate

METHODS

Total Reducing Sugars

The Hanes (4) modification of the Hagedorn and Jensen method for total reducing sugars was adapted as follows: 5 cc. of solution "A" were added to 10 cc. of the reducing sugar solution (containing 0.5 mg. to 3.0 mg. of reducing sugar) in a 30×160 mm. Pyrex test tube, covered with a glass funnel, and placed in a boiling water bath for 15 minutes. The solution was then placed in the cooling bath for 3 minutes, after which 5 cc. of solution "B" and 3 cc. of solution "C" were added. The iodine liberated was then titrated with standard sodium thiosulphate (solution "E"), using 3 drops of the starch solution (solution "D") as the indicator.

A blank was run each day using 10 cc. of distilled water in place of the sugar solution. The difference in the titrations (W.B. −S) was changed to the equivalent in 0.01 N thiosulphate. Substituting this titration value into the formula given by Hulme and Narain (6),

$$\text{sugar (mg.)} = b(T+a)$$

in which T is the titration difference (W.B. −S) and a is 0.05 cc. for all the sugars investigated; the value of the factor b, significant of the reducing power of the different sugars, was determined.

The factors b, determined by Hulme and Narain (6) for glucose and fructose, were 0.340 and 0.341 respectively. The factors calculated in this report were 0.340 for glucose and 0.336 for fructose. The close agreement of the calculated factors justifies the use of the factors 0.378 for arabinose and 0.358 for xylose, determined in the same manner (Table I). These factors were redetermined each day to avoid the error caused by the changing concentrations of the reagents.

Aldose Determination

Macro-determination. With large amounts of aldose sugar (20–60 mg.)

good results were obtained by using the Willstätter (12) iodimetric procedure. To 30 cc. of the sugar solution containing 20–60 mg. of aldose, 10 cc. of 0.1 N iodine in potassium iodide were added and then 22.5 cc. of 0.1 N sodium hydroxide during four minutes. The mixture was allowed to stand for 15 minutes at room temperature, then acidified with 10 per cent sulphuric acid (about 6 cc.), and titrated with 0.1 N sodium thiosulphate.

TABLE I

DETERMINATION OF FACTORS FOR CALCULATING TOTAL REDUCING SUGARS

Weight of sample, mg.	Sugar	Observed titration 0.0128 N thiosulphate solution, cc.	0.0128 N alkaline ferricyanide reduced, cc. (blank-obs. titration)	T, 0.01 N thiosulphate equivalent of reduced ferricyanide, cc.	Corrected 0.01 N thiosulphate equivalent, cc. T+a	Factor, $b=\frac{\text{mg. sugar}}{\text{T}+a}$
0	Blank	9.37	0.00	0.00	0.05	—
2	Glucose	4.81	4.56	5.84	5.89	0.340
2	Fructose	4.76	4.61	5.90	5.95	0.336
2	Arabinose	5.28	4.09	5.24	5.29	0.378
2	Xylose	5.05	4.32	5.53	5.58	0.358

A water blank was run at the same time. The difference between the sugar titration and the water blank (W.B. −S), multiplied by 9.0 mg. per cc. in the case of hexoses, or 7,505 mg. per cc. in the case of pentoses, gives the weight of the aldose sugars present in milligrams. In Table II a representative run made to determine the effect of fructose on the glucose determination is shown using this method. As may be seen, the error caused by the presence of fructose in a fructose-glucose mixture is less than 4 per cent. When fructose was tested alone, a very small titration difference from the water blank was obtained which was not strictly additive in aldose mixtures as shown in Table II.

TABLE II

EFFECT OF THE PRESENCE OF VARYING AMOUNTS OF FRUCTOSE ON 50 MG. OF GLUCOSE AS DETERMINED BY THE WILLSTÄTTER HYPOIODITE PROCEDURE

Weight of fructose, mg.	Observed titration 0.116 N thiosulphate solution, cc.	Equivalent of 0.100 N thiosulphate	Equivalent of 0.10 N I_2 reduced by sugars, in cc.	Apparent glucose, mg. cc.×9.0
Water blank	8.39	9.74	—	—
0.0	3.60	4.18	5.56	50.0
5.0	3.52	4.08	5.66	50.9
10.0	3.53	4.09	5.65	50.8
15.0	3.63	4.21	5.53	49.7
20.0	3.59	4.17	5.57	50.1
25.0	3.59	4.17	5.57	50.1
30.0	3.59	4.17	5.57	50.1
35.0	3.55	4.12	5.62	50.5
40.0	3.43	3.98	5.76	51.8
45.0	3.44	3.99	5.75	51.7
50.0	3.43	3.98	5.76	51.8

Micro-determination. When the amount of aldose sugar was in the range of 0.5 mg. to 3.0 mg., an adaptation of the iodimetric procedure suggested by MacLeod and Robison (7) was followed. The sugar solution was added to 3 cc. of 0.02 N iodine and diluted to 6 cc. There was then slowly added 0.4 cc. of 5 per cent sodium carbonate solution and the mixture was allowed to stand at 21° C. for 30 minutes. The solution was acidified with 1 cc. of 0.5 N sulphuric acid and titrated with 0.005 N sodium thiosulphate. As in the previous method, a water blank was run at the same time and each cc. of difference (W.B. −S) was equivalent to 0.450 mg. of hexose or 0.375 mg. of pentose.

Using these factors, the titration equivalents of 1 and 2 mg. samples of each sugar were determined. The resulting calculations showed apparent glucose to exceed 95 per cent of that known to be present, xylose and arabinose in excess of 97 per cent, and fructose, the only ketose sugar tested, less than 4 per cent. The accuracy of the micromethod was thus shown to be well within the limits claimed by the originators of the method.

Fermentation of Hexoses

Experiments were first run to determine the rate of fermentation of glucose, fructose, arabinose, and xylose. One cake of Fleischmann's yeast (approximately 20 g.) was alternately suspended in 50 cc. of distilled water and centrifuged, until the supernatant liquid remained clear. This was usually accomplished after about four washings.

The water-extracted yeast was then again suspended in 50 cc. of distilled water and various volumes filtered through Celite. The clear filtrate was tested for reducing power and the titration expressed as apparent glucose. This value was thus found to be 0.03 mg. per cc. of the yeast filtrate. Accordingly this correction was applied to all calculations involving the use of the yeast suspension.

Since it was assumed that fermentation ceased as soon as the suspended yeast was filtered from the sugar solution, it was of importance to determine whether or not the filtered yeast extract retained any fermenting action on the various sugars. That this assumption was correct was shown by adding 5 cc. aliquots of the filtered yeast extract to 5 cc. samples of sugar solution, each containing 2 mg. of glucose, allowing them to stand at room temperature for various lengths of time, and then testing for reducing sugar. A constant titration over a period of two hours, which was equivalent to 2.15 mg. of apparent glucose, established the simple additive nature of the reducing value of the 5 cc. aliquots of filtered yeast extract (5 cc.×0.03=0.15 mg.) to that of the glucose (2.00+0.15=2.15 mg.).

The selective action of yeast on sugar mixtures has been shown by Raymond and Blanco (8). The four sugars used in this report were run separately to determine rates of fermentation. The results shown in Figure 1 indicate three hours as the minimum time in which glucose and fructose can be eliminated by fermentation from a mixture of these hexoses with pentoses.

During the three-hour period of fermentation 25 per cent of each of the pentoses is also eliminated (Table I and Fig. 2). In mixtures with

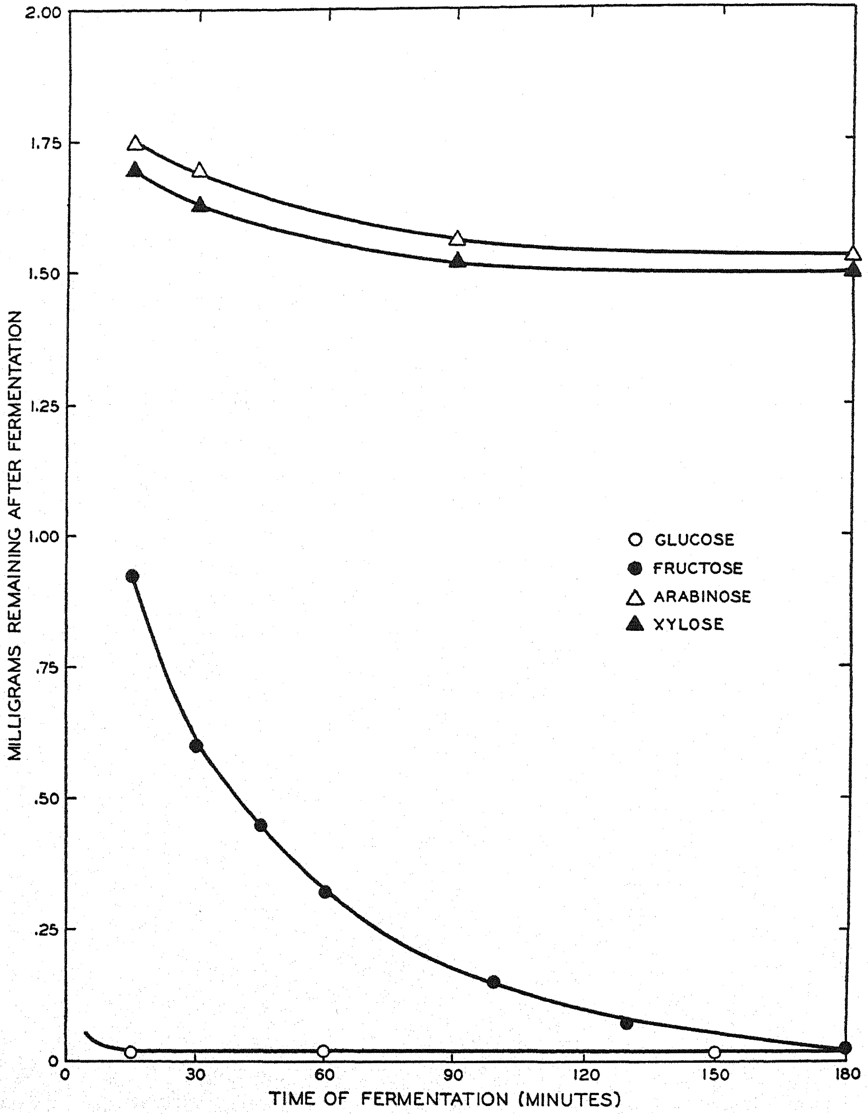

FIGURE 1. Rate of yeast fermentation of 2-mg. samples of glucose, fructose, arabinose, and xylose over a period of three hours at room temperature.

hexoses, however, the fermentation of the pentoses was reduced to approximately 15 per cent.[2] The variance in percentage of apparent pentose

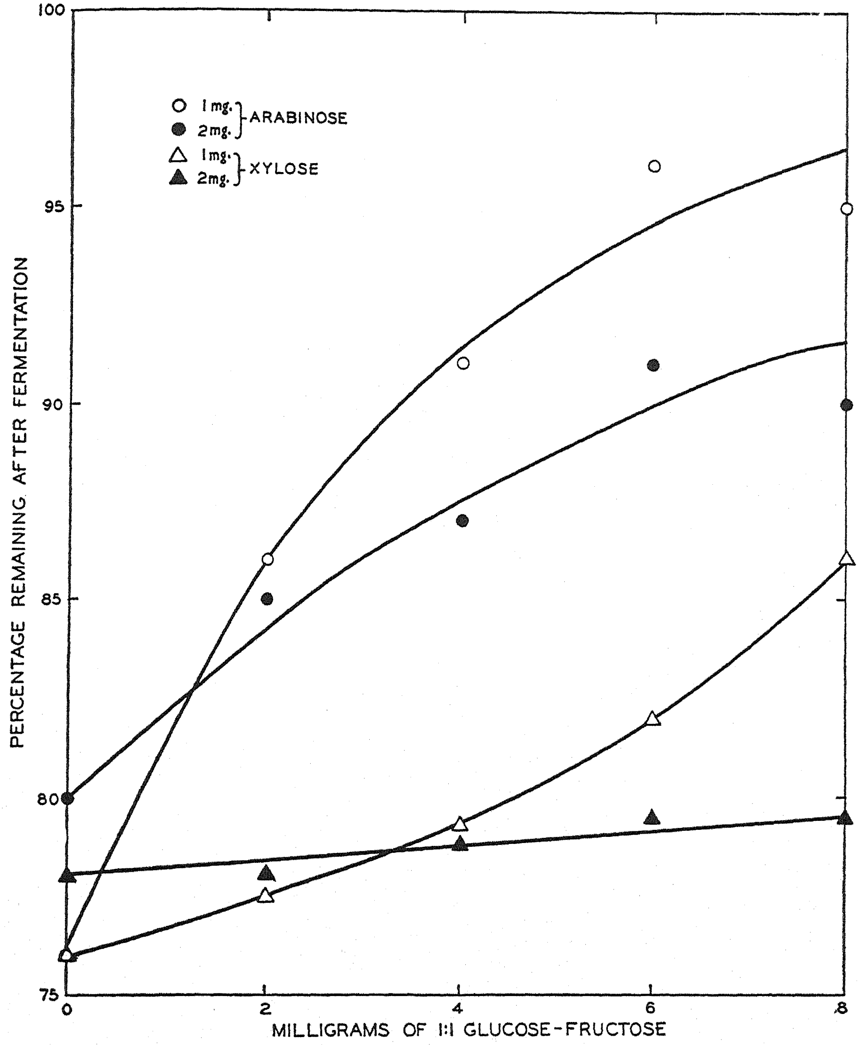

FIGURE 2. Effect of concentration of 1:1 glucose-fructose mixture on the percentage of 1- and 2-mg. samples of arabinose and xylose remaining after three hours of yeast fermentation at room temperature.

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[2] After the approximate amount of apparent pentose has been determined in simple mixtures containing a known pentose, i.e., arabinose or xylose, the value of 15 per cent may be varied from inspection of Figure 2 if greater accuracy is desired. Although the curves given in Figure 2 were determined with a glucose to fructose ratio of 1:1, the results will be accurate within the limits of 5 per cent for any other ratio.

present after three hours' fermentation, as a function of the pentose concentration, is also shown in Table III and Figure 2. The range of sugar concentrations in this fermentation process is 0.0 to 10.0 mg. of hexoses and 0.5 to 3.0 mg. of pentoses.

TABLE III

Effect of the Presence of Glucose and Fructose on the Rate of Yeast Fermentation of Arabinose and Xylose

Components of arabinose-glucose-fructose mixture			Apparent arabinose after fermentation for 3 hours, mg.	Components of xylose-glucose-fructose mixture			Apparent xylose after fermentation for 3 hours, mg.
Arabinose, mg.	Glucose, mg.	Fructose, mg.		Xylose, mg.	Glucose, mg.	Fructose, mg.	
1.00	0.00	0.00	0.76	1.00	0.00	0.00	0.76
2.00	0.00	0.00	1.60	2.00	0.00	0.00	1.58
1.00	1.00	1.00	0.86	1.00	1.00	1.00	0.73
1.00	2.00	2.00	0.91	1.00	2.00	2.00	0.79
1.00	3.00	3.00	0.96	1.00	3.00	3.00	0.82
1.00	4.00	4.00	0.95	1.00	4.00	4.00	0.86
2.00	1.00	1.00	1.70	2.00	1.00	1.00	1.56
2.00	2.00	2.00	1.74	2.00	2.00	2.00	1.57
2.00	3.00	3.00	1.81	2.00	3.00	3.00	1.59
2.00	4.00	4.00	1.80	2.00	4.00	4.00	1.59

QUANTITATIVE DETERMINATION OF GLUCOSE AND FRUCTOSE IN THE PRESENCE OF PENTOSE

The sugar mixture was dissolved and diluted to a volume such that the solution contains about 2 mg. per cc. of reducing sugar. The analysis was resolved into three steps as follows:

1. Total reducing sugars in 1 cc. of the solution were determined by means of the Hanes (4) modification. The resulting titration after correction is represented by A in the Sample Calculations.

2. Aldose sugars were determined in either of two ways. (a) Thirty cc. of the sugar solution were tested by the Willstätter hypoiodite titration (12). This titration, after correction, was equivalent to the amount of glucose plus pentose in the solution. Since the pentose factor is 7.505 mg. per cc., and the hexose factor 9.00 mg. per cc., it is impossible to determine either sugar quantitatively at this point, unless the proportion of each in the mixture is known. This titration will accordingly be represented simply as B in the Sample Calculations. (b) Two cc. of the sugar solution were tested, using the MacLeod and Robison (7) adaptation of the iodimetric method to micro-technique. This titration is proportional to B and will be represented by B_m in the Sample Calculations. This method, while not quite as accurate as the first, was found to be satisfactory.

3. Hexoses were removed from a 1 cc. aliquot of the sugar solution by diluting to 10 cc. and adding 3 cc. of yeast suspension. After standing with

frequent shakings for three hours, the solution was filtered through Celite and analyzed as described in step 1 (above) for remaining reducing sugars. The titration value after correction was divided by 0.85 (since 15 per cent of the pentose content was fermented in three hours) and is represented by C in the Sample Calculations. The value of 0.85 may be changed according to Figure 2 after an approximation has been made.

It now follows that $(A-C)\times 0.338$ is equal to fructose plus glucose in mg. per cc. Also, $(C\times 0.368)$ is equal to pentoses present in mg. per cc. Then $30(C\times 0.368)$ divided by the pentose factor (7.505 mg. per cc.) from 2(a) gives that part of the aldose titration B reduced by the pentose, in cc. It is then possible to determine, by subtracting this value from B, the milligrams of glucose in 30 cc. (glucose factor equals 9.00 mg. per cc. of solution). Similarly the titration obtained by the second method, 2(b) may be calculated, using the proper factors, to the glucose equivalent in mg. per cc. The fructose content of the solution in mg. per cc. was then obtained upon subtracting the mg. of glucose per cc. from $(A-C)\times 0.338$.

Sample Calculations

In a typical example the observed titrations, after applying the various corrections previously outlined, gave the following values:

$A = 7.00$ cc. 0.1 N ferricyanide solution reduced
$B = 6.00$ cc. 0.1 N iodine solution reduced
$B_m = 8.03$ cc. 0.005 N iodine solution reduced
$C = 3.00$ cc. 0.1 N ferricyanide solution reduced

The value of the factors for the various sugars were:

Hypoiodite aldose titration
  Pentoses = 7.505 for 0.1 N iodine solution or 0.375 for 0.005 N iodine solution
  Aldohexoses = 9.00 for 0.1 N iodine solution or 0.450 for 0005 N iodine solution
Ferricyanide reducing sugar titration
  Pentoses = 0.368 (average of arabinose and xylose factors) (Table I)
  Hexoses = 0.338 (average of glucose and fructose factors) (Table I)

Pentose Calculation

$$C\times 0.368 = 3.00 \text{ cc.}\times 0.368 = 1.104 \text{ mg. per cc.}$$

Hexose Calculation (glucose+fructose)

$$(A-C)\times 0.338 = 4.00 \text{ cc.}\times 0.338 = 1.35 \text{ mg. per cc.}$$

Aldohexose Calculation (glucose)

From procedure 2(a) above employing 30 cc. of reducing sugar solution:

No. of cc. of 0.1 N ferricyanide reduced by pentoses in 30 cc.

$$B' = \frac{30.00 \text{ cc.} \times 1.104 \text{ mg./cc.}}{7.505} = 4.41 \text{ cc.}$$

Then $(B - B') \times 9.0$ = mg. of glucose in 30 cc. of solution.

1.59 cc. $\times$ 9.0 = 14.31 mg. glucose in 30 cc. solution

$$\text{or } \frac{14.31}{30} = 0.48 \text{ mg. per cc.}$$

Similarly, procedure 2(b) above, employing 2.00 cc. of reducing sugar solution, gives the same value.

No. of cc. of 0.005 N ferricyanide reduced by pentoses in 2 cc.

$$B'' = \frac{2.00 \text{ cc.} \times 1.104 \text{ mg./cc.}}{0.375} = 5.89 \text{ cc.}$$

Then $(B_m - B'') \times 0.450$ = mg. glucose in 2 cc. solution.

8.03 cc.–5.89 cc. = 2.14 cc. $\times$ 0.450 = 0.96 mg. in 2 cc.

$$\text{or } \frac{0.96 \text{ mg.}}{2.0 \text{ cc.}} = 0.48 \text{ mg. per cc.}$$

Ketohexose Calculation (fructose)

Hexose – aldohexose, mg. per cc. = mg. of fructose per cc. of solution

1.35 mg./cc. – 0.48 mg./cc. = 0.87 mg. of fructose per cc.

From the above determined values the percentage of each sugar constituent may be calculated. Thus the pentoses are 45.0 per cent, glucose, 19.6 per cent, and fructose, 35.4 per cent.

SUMMARY

1. The quantitative yeast fermentation of glucose can be accomplished in less than fifteen minutes, and fructose in three hours. Arabinose and xylose are fermented to the extent of approximately 25 per cent in three hours.

2. As the amount of hexose in a hexose-pentose mixture increases, the percentage of pentose fermented decreases.

3. A method has been devised for the quantitative determination of glucose and fructose in mixtures with pentoses.

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RESPIRATION OF GLADIOLUS CORMS DURING PROLONGED DORMANCY

F. E. Denny

That the dormancy of gladiolus corms could be prolonged for many months by the simple expedient of planting the *freshly-harvested* corms in *moist soil* and storing them at *room temperature* was shown in a previous report (1). Corms of many (but not all) varieties remained in the soil for periods of 6 to 12 months under such conditions without sprouting. A test made after nearly seven months' storage showed that the corms were still in good condition, that the dormancy could be broken easily by treatment with vapors of ethylene chlorhydrin, and that good growth of plants with apparently normal blooms resulted.

It is true that with some varieties corms stored for as much as one year before dormancy-breaking treatments were applied gave a spindling type of growth, but a continuation of the experiment with additional varieties has shown that this was not the general situation, but that at least many varieties will respond with satisfactory growth and blooms after about a year's storage in moist soil at room temperature.

Furthermore, corms of varieties harvested in October 1937 after being stored under these conditions without sprouting until May 1939 when removed from the soil were found to be plump and sound. Since corms will remain in such good condition over such a long period at room temperature it would seem that their respiration must have been much retarded during that period. It was to obtain measurements on this point that the experiments here reported upon were undertaken.

The results showed that the respiration of the corms was, indeed, very low, being only about 2 to 7 mg. of CO_2 per kg. per hour, i.e., not more than, and probably less than, 10 per cent of the rate of non-dormant corms. But the most interesting feature of the experiments was in the series of changes in respiration rate undergone by these dormant corms in the first few hours after their removal from the soil. After about four hours the respiration rate began to rise, and within about 24 hours had increased 5-fold, 10-fold, 20-fold, or perhaps even more. Yet, this proved to be only a temporary increase, and there followed a gradual decrease, so that after about four days the curve tended toward, but did not reach, the original low values. If replanted in the soil for about three weeks and again removed for a respiration measurement, the rate was again found to be low, but this time the large increase in rate during the 24-hour period after removal from the soil did not ensue, the rate increasing only a little or not at all.

These changes in respiration rate occurred without change in temperature. And the increase in respiration rate was not accompanied by sprouting of corms, these maintaining their dormancy during all of the tests and for at least many weeks thereafter until the time of the writing of this report.

METHODS

The respiration tests were begun February 28, 1939. For the most part the corms used were from the 1938 crop, harvested in October 1938, allowed to cure in air for about a week after harvest, after which the corms were cleaned by the removal of the roots, old corm, and cormels, were planted without removal of husks, at once in moist soil in flats. These flats were piled in stacks about four feet high and were stored at room temperature, which varied from about 21° C. to about 25° C. The flats were examined weekly and water applied as needed to keep the soil moist. One test was carried out with bulbs of the 1937 crop carried over from October 1937 in the manner described for the 1938 corms.

The respiration measurements were made in a thermostatically controlled room the temperature of which was adjusted to be equal to the temperature of the soil in which the corms were stored, this temperature being taken by a thermometer inserted in the soil on the day a respiration test was to be made. The stacks of flats, being arranged close to each other, maintained the temperature rather uniformly, so that only small and gradual changes in temperature occurred.

Glass desiccators of about three-liter capacity equipped with a glass tubing inlet at the top and an exit tube from the bottom served as containers for the corms during the respiration tests. The number of corms used in each test varied from 25 to 50, and the weights from about 400 to 1000 g. The carbon dioxide formed in the desiccators by the sample of corms was swept out by a current of air and was absorbed in a solution of barium hydroxide in Van Slyke-Cullen tubes. The procedure was that previously described by Miller (2). An empty desiccator of the same size as those containing corms served as a control. Air was drawn through it and through $Ba(OH)_2$ tubes in a manner identical with that employed with desiccators containing corms. The titration value of this control was used as the basis for computing the respiration rates.

RESULTS

The respiration rate of corms that were removed from the soil and placed in glass desiccators in a current of air is shown in Figure 1 for a period up to 100 hours after removal from the soil. The CO_2 was absorbed during four-hour periods and the value so obtained was plotted at the time period represented by the middle of the respiration-test period. It is seen that during the first four hours the rate was very low, being in these three cases 2.7, 5.2, and 6.9 mg. of CO_2 per kg. of tissue per hour (reading the curves of Fig. 1 in the order bottom to top). Maintaining the current of

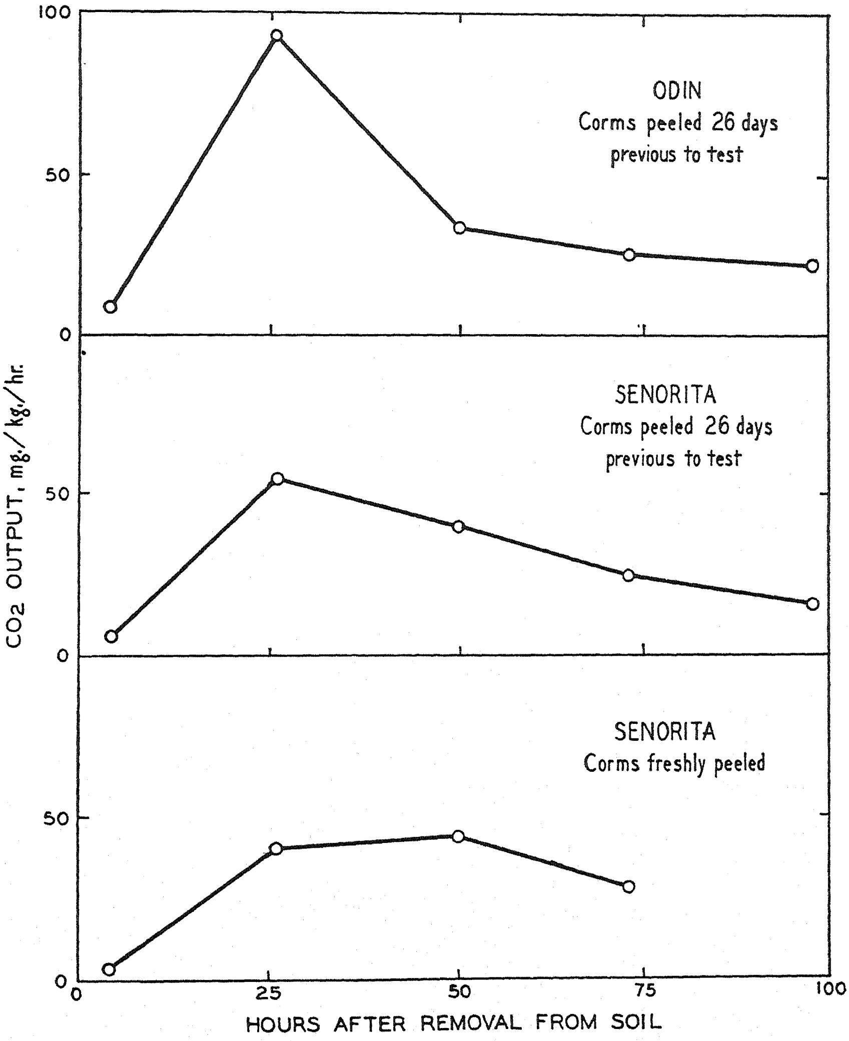

FIGURE 1. Respiration of gladiolus corms harvested in the period from September 28, 1938 to November 1, 1938, placed in moist soil within about one week from time of harvest, and stored in moist soil at room temperature until the period of these experiments February 28, 1939 to May 26, 1939. Then corms were removed from soil and placed in containers for determining rate of CO_2 production. Bottom curve: Senorita, corms freshly-peeled and placed in respiration chamber; middle and top curves: corms peeled 26 days previous to respiration test, returned to soil at once after peeling, and not removed from soil until time of respiration test (middle curve, Senorita; top curve, Odin).

air through the desiccators and making another measurement during the period 24 to 28 hours after the start of the experiment, a large rise in the respiration rate occurred, the rate reaching 40.2, 57.6, and 92.1 mg./kg./hr. Still continuing the air current the rate then decreased but even after about 100 hours was still somewhat larger than during the first four hours after removal from the soil. On the Senorita freshly-harvested corms (lower curve in Fig. 1) a test was made at a later period than that shown in Figure 1, i.e., during the period 168 to 172 hours after the start and the value found was 16.6 mg. CO_2/kg./hr., being lower than at the 96th-100th hour but still considerably higher than at the beginning.

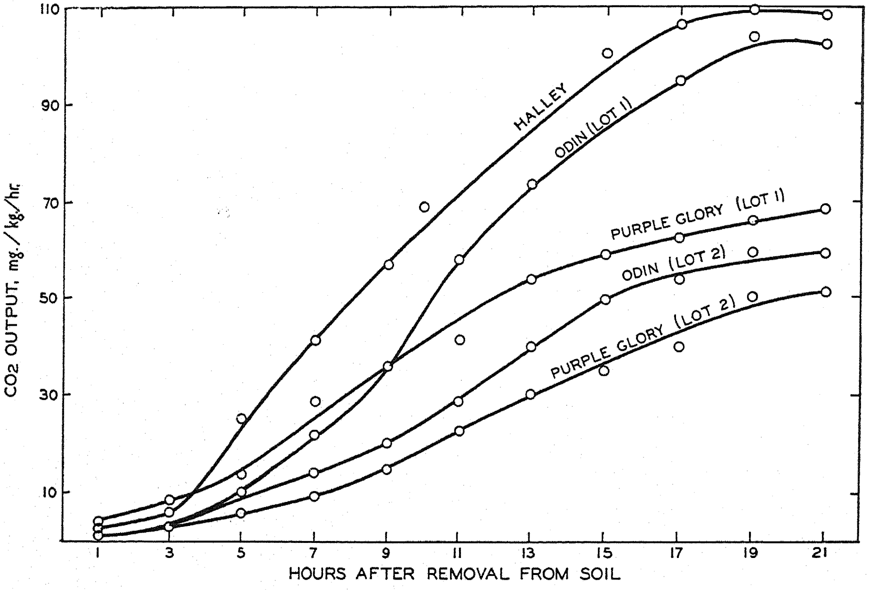

FIGURE 2. Respiration of gladiolus corms during the first few hours after removal from the soil in which the dormancy of the corms had been prolonged for 5 to 6 months by the conditions of harvest and storage described in the legend of Figure 1. Halley, corms freshly-peeled; Odin (Lot 1), corms freshly-peeled; Odin (Lot 2), corms not peeled; Purple Glory (Lot 1), corms peeled 41 days previously and peeled corms returned at once to soil and not removed until the respiration test was to be started; Purple Glory (Lot 2), freshly-peeled.

Another experiment was run to determine the form of the curve during the first few hours, and in particular to catch the time at which a marked increase in rate occurred. The results are shown in Figure 2. In this case a continuous record was obtained, the CO_2 being collected during each two-hour period, and the values so obtained were plotted at the middle of each period, i.e., at 1, 3, 5, etc. hours after removal from the soil.

During the first four hours the change in respiration rate was small or not detectable, but beginning at about that time, or at least in the eighth to tenth hour, the increase began, the rise in the curve being quite rapid for the Halley corms and slower for the freshly-peeled Purple Glory corms. Another lot not shown in Figure 2 but included in the tests was that of the variety Dr. F. E. Bennett, corms freshly-peeled, these showing a curve quite similar to that of the previously peeled Purple Glory corms in Figure 2. By the eighteenth to twentieth hour, four of the six lots in the experiments had reached their maxima and since there was evidence that with the other two not much higher values could be expected the experiment was discontinued. The ratios of the final rates (at the 20th to 22nd hour) to the initial rate (during the first two hours) were for the six experimental lots as follows: 23, 11, 52, 38, 15, 29. Ratios obtained in a similar way for the lots shown in Figure 1 (26th hour value divided by the 2nd hour value) are 15, 11, 13. This gives an average increase of 23-fold during a period of about 24 hours.

EFFECT OF PEELING THE CORMS

When duplicate samples of corms of the same variety were taken for the respiration test, one lot having the husks removed at once after the corms were taken from the soil, the other lot being placed at once into the respiration chambers without removal of husks, it was found that for the first four to six hours the rates of CO_2 production were about the same in the two cases. But after that time the increase in respiration was greater for the lot with husks removed than with that having husks intact, and the maximum rate reached after about 24 hours was greater for the corms with husks removed.

However, by the use of corms previously peeled (husks removed), returned to the soil, allowed to remain there for a considerable period, and then removed from the soil for the respiration test, it was found that the increase in respiration rate during the first 24 hours was as great or greater with corms previously peeled as with those freshly-peeled. This is shown in both Figure 1 and Figure 2. For example, in Figure 1, Senorita bulbs peeled on March 8 and returned to the soil at once after peeling and not removed from the soil until April 3, gave a respiration increase somewhat higher than corms of the same lot peeled just before being placed in the respiration chambers. This same result is shown also by the two lots of Purple Glory corms in Figure 2. With the Odin lot, however, in Figure 1 the increase was not quite as great as was found in the corresponding lot in Figure 2.

THE CONTAINER AS A FACTOR

In the experiments the results of which are shown in Figures 1 and 2, the stream of air was led continuously through the respiration chambers, and the question arose whether this condition was a necessary factor in the respiration increase during the first 24-hour period. To get a comparison with corms subjected to this procedure, a second sample of corms from the same lot was placed not in the respiration chamber but on a glass plate, the corms being spaced widely apart and covered loosely with a piece of moist cloth adjusted in such a way as to allow air drainage at the edges of the glass plate. After 24 hours these second lots were placed in respiration chambers, and the rate for each lot was determined. The results were as follows: variety Princeps, chamber 77.0, glass plate 70.0; variety Senorita, chamber 47.5, glass plate, 43.2; variety Purple Glory, chamber 49.4, glass plate 42.4. The higher rates were shown by the lots continuously in the chamber, but the data show that the conditions in the chamber were not necessary for the establishment of a high respiration rate at the end of a 24-hour period after the removal of the corms from the soil.

RESPIRATION OF CORMS FROM THE 1937 CROP

Some bulbs of the varieties Golden Measure, Halley, Senorita, and Purple Glory had been held in a dormant condition in moist soil from the 1937 crop, harvested in October 1937. These were removed from the soil on April 10, 1939, the husks were removed, and the respiration was found to be 8.5 mg./kg./hr. The stream of air through the respiration chamber was continued until the next day and the rate during the 24th to 28th hours was found to be 41.0 mg./kg./hr. These corms, therefore, after being held in a dormant condition for about one and one-half years behaved, so far as their respiration rate was concerned, in a manner similar to corms held for only five to seven months; their respiration rate was low when first removed from the soil, and the rate had increased considerably by the end of one day thereafter, although it may be that this increase was less with the 1937 corms than with those of the 1938 crop.

SECOND TESTS ON CORMS REPLANTED IN SOIL AFTER THE FIRST TEST

Corms which had been removed from soil and had been found to respond in the manner described in the previous pages, i.e., by showing a low rate of respiration when first removed from soil and showing a 5-fold to 20-fold increase in respiration within about 24 hours thereafter, when replanted in soil and allowed to remain undisturbed for three to five weeks, and again removed from the soil for a respiration measurement, behaved quite differently on this second test. The respiration rate was low, although not quite so low as in the original test, but the large increase in respiration by the end of a 24-hour period so characteristic of the first test did not occur in the second test. The increase in rate was small, or at least not comparable in extent to that found in the original test on the same corms. For example, Odin corms, which in the first test showed an increase from 4.4 mg. to 95.5 mg. CO_2/kg./hr., when replanted in soil for 28 days and again removed showed an increase from 8.9 to 24.4 mg. CO_2/kg./hr. For Purple Glory the values on the first test were 6.6 to 48.4 mg. CO_2/kg./hr., and on the second test after 40 days in soil the values were 13.9 to 14.2 mg. CO_2/kg./hr. For Senorita the corresponding values were 3.4 to 52.5 mg. CO_2/kg./hr. on the first test, and 10.1 to 17.7 mg. CO_2/kg./hr. on the second test after the corms had been replanted for 24 days in the soil.

Although the tests just described showed that an interval of four to six weeks in the soil following a first respiration test was insufficient to permit the corms to respond with a large gain in respiration when the corms were removed from the soil for a second test, some evidence was obtained that if the interval in the soil was as long as three months after the first test a large gain would result in the second. Corms of the variety Senorita from the same lot of corms that was used in the respiration tests were available from a previous experiment not connected with the respiration of the corms. The corms had been maintained in moist soil from November 1, 1938 until February 16, 1939 on which date they had been removed from the soil, had been stored for 48 hours in a paper bag in air, and had then been replanted in soil. The corms were again removed from the soil on May 25, 1939, and the respiration during the first three hours after removal from the soil was found to be 2.9 mg. CO_2 per kg. per hr. during the three-hour period from the 24th to 27th hours, however, the rate was found to be 97.0 mg. CO_2/kg./hr., showing an increase in respiration of about 30-fold. This increase in respiration was larger than any previously observed with corms of this variety, and furnishes evidence that if the sojourn in soil after the first removal from the soil is long enough, then the large increase in respiration characteristic of corms on the first test after removal from soil can occur also upon a second test.

SPROUTING BEHAVIOR OF CORMS REPLANTED IN SOIL AFTER RESPIRATION TESTS WERE COMPLETED

After the respiration tests were completed the corms were replanted in soil in flats which were stacked in tiers, stored at room temperature, and examined at weekly intervals to maintain the proper moisture in the soil. Although the respiration of the corms had increased many-fold at some stage during the tests, this increase was not accompanied by sprouting of corms. Occasional sprouts were produced, especially with corms of the variety Odin, but, on the whole, the corms have retained their dormant condition, and are being maintained in a dormant state for subsequent tests.

SUMMARY

Gladiolus corms maintained in a dormant condition for 5 to 18 months by being replanted in moist soil soon after harvest and being stored in a

moist condition at room temperature were found to show a very low rate of carbon dioxide production during the first four hours after removal from the soil.

Immediately or at least soon thereafter the respiration rate rose and reached a maximum about 20 hours later, at which time the increase in CO_2 output was found to be 5-fold, 10-fold, 30-fold, or even larger.

The rate then decreased until after about five days it approached but did not reach the low values shown by the corms at the time of removal from the soil.

If replanted in the soil the respiration rate again returned to low values, and if this second sojourn in the soil was only about four to six weeks before the corms were again removed for a respiration test, the large gain in respiration during the 24-hour period after removal from the soil so characteristic of the corms on the first test did not occur in the second test. However, when the sojourn was as long as three months the large gain in respiration was again observed.

These changes in respiration rate were obtained without change in temperature.

Corms whose respiration had been increased many-fold at some stage of the tests when replanted in soil did not sprout, but have continued to maintain their dormant condition.

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COMPARATIVE ACTIVITY OF ROOT-INDUCING SUBSTANCES AND METHODS FOR TREATING CUTTINGS

A. E. Hitchcock and P. W. Zimmerman

INTRODUCTION

Experimental work extending over several years has resulted in the development of a number of methods for treating cuttings with root-inducing substances. Although each method has proved effective at least on certain plants, there has been a tendency to work toward more simplified procedures for use in practice. Modified methods in which the duration of treatment is reduced to a minimum of a few seconds simplifies application of the root-inducing substances, but introduces complications associated with the use of concentrations 10 to 1,000 times higher than those used for the standard 24-hour treatment (3).

Results with powder preparations are complicated by the fact that the carrier most commonly used (talc) showed some activity itself and thus consistently caused better rooting than was obtained on non-treated or tap water controls. In addition, the effectiveness of powder preparations was noticeably dependent upon the mechanical fineness of the powder. For this reason the results of different workers may not always agree unless strictly comparable preparations are used.

It is the purpose of the present paper to present the results of comparative tests making use of several methods of subjecting cuttings to the action of root-inducing substances. Results with powder preparations (0.5 to 50 mg./g.) and with solution concentrates (1 to 20 mg./cc.) showed that different dosages were required for different kinds and types of cuttings just as in the case of the standard 24-hour treatment with 1 to 80 mg. per liter. Whichever method is used, of the three just mentioned, the root-inducing substance must first be in solution before it can enter the tissue. It has not been determined how vapors enter the tissue. The powder preparations were more effective when the basal ends were moistened before being dipped into the powder.

MATERIALS AND METHODS

Indolebutyric, naphthaleneacetic, and indoleacetic acids and their potassium salts were the principal root-inducing substances used. In one test the methyl esters of naphthaleneacetic and indolebutyric acids were applied as vapors to the cuttings for a period of 30 minutes, in a bell jar, and then the cuttings were planted as described below. The species of cuttings used are listed in Table I. Other species treated with powders only, appear in the text.

Standard solution immersion method. Basal ends of cuttings were immersed for 24 hours in water containing the root-inducing substance in concentrations of 1 to 80 mg./l. (milligrams per liter of water). Control lots were immersed in tap water for the same period. After treatment the cuttings were planted in greenhouse benches or in cold frames.

Concentrated solution dip method. Solutions containing the root-inducing substance in concentrations of 1 to 20 mg./cc. (milligrams per cubic centimeter) were used in these tests. The solvent was either water, alcohol-water mixtures, or diethyleneglycol. Basal ends of the cuttings were dipped into the solution to a depth of about one-half inch. Control cuttings were similarly dipped into tap water. After treatment the cuttings were planted in greenhouse benches or in cold frames.

Powder dip method. Basal ends of cuttings were dipped to a depth of about one-half inch in powders containing the root-inducing substance. The method of dipping had to be modified slightly according to the number of cuttings which were treated simultaneously. Thus to secure uniform coating of the basal part of the stem it was necessary to stir the immersed parts to the extent of one to two revolutions in cases where a group of cuttings was treated. The cuttings were then tapped gently on the rim of the container in order to remove excess powder. Since in preliminary tests the dipping of cuttings with dry stems proved relatively ineffective for most species, the final method adopted was to wet the basal ends of the cuttings just before dipping them into the test powders. Control cuttings were similarly wetted and then dipped in talc to which a root-inducing substance had not been added.

Two types of powders were used in the tests. In one case a commercial grade of talc (Eimer and Amend) and the root-inducing substance were mixed in the proportions of 1, 4, 10, 25, and 50 mg. of the root-inducing substance per gram of talc. All of the root-inducing substances mentioned in the first part of this section were obtained from Merck & Co. Inc., Rahway, New Jersey. A second series of powders prepared by Merck & Co. Inc., contained the root-inducing substances in various concentrations ranging from 1 to 35 mg./g. In order to avoid confusion, exactly the same concentrations in the two series are not reported in this paper, even though all concentrations were used originally. Thus concentrations of 2, 5, 6, 12, 18, and 35 mg./g. have been used to designate powders prepared by Merck & Co. Inc. Merck powders tested, but for which specific data (with one exception) are not presented, were as follows: 0.5, 1, 3, 8, 10, and 16 mg./g. The one exception is the 1 mg./g. powder which was used on the Briarcliff rose (Fig. 1 A) and chrysanthemum (Table II). The Merck powders appeared to be of a finer texture than the powders which were prepared in our laboratory.

As in the case of the other methods, the cuttings were planted immediately after treatment with the test powders.

RESULTS

CONCENTRATION REQUIREMENTS ACCORDING TO THE METHOD OF TREATING THE CUTTINGS

Data in Table I show that the optimum concentration of indolebutyric acid varied with the species regardless of whether the root-inducing substance was applied as a relatively dilute solution, as a concentrated solution, or as a powder. Species such as the Briarcliff rose (Fig. 1 A and Table III), begonia, ivy, and chrysanthemum (Table II) responded to treatment with relatively low concentrations with all three methods. Likewise, certain species responding best to relatively high concentrations with one method also required relatively high concentrations with the other two methods. Some of the species falling in the latter class were *Thuja occidentalis* var. *robusta*, *Chamaecyparis obtusa* var. *filiformis*, *Rhododendron* (Fig. 2, A and C), *Tsuga canadensis* (Fig. 3 A), *Abies veitchii*, *Syringa vulgaris* (Table IV and Fig. 2 B), *Picea glauca* var. *conica*, and *Camellia* (Fig. 2 D). Species requiring intermediate concentrations were carnation (Fig. 1 B), dahlia, *Prunus*, elm, *Celastrus*, and *Caragana*.

Concentrated solutions and powders containing indolebutyric acid were about equally effective on a weight basis of root-inducing substance per gram of carrier (Table I), but the latter values are 10 to 1,000 times the concentrations (by weight) which induced equivalent rooting when applied according to the standard immersion method of treatment. The concentrated solution dip method was generally more critical than the other two methods but the results obtained with the former were particularly satisfactory in the case of species difficult to root such as the apple (Fig. 2 E), hemlock (Fig. 3 A), rhododendron (Fig. 2, A and C), and many of the evergreens listed in Table I.

Although the response of *Pachysandra* (Fig. 1 C) and *Hibiscus* (Fig. 1 D) indicates tolerance to a relatively wide range of concentrations, it is to be noted that concentrations of 18 to 35 mg./g. caused toxic effects consisting of the production of an excessively large number of roots, or only a few roots, failure to root, excessive proliferation, the retardation or inhibition of bud growth, or killing of the lower part of the stem. Thus for *Pachysandra* the optimum concentration was between 5 and 12 mg./g. and for *Hibiscus* between 6 and 18 mg./g. Since lower concentrations were effective in producing more roots than on control cuttings, these two species represent tolerant types. Most of the other species listed in Table I were not so tolerant.

The same correlation between the average number of roots per cutting and concentration as was previously reported for the standard immersion method of treatment (4) was also found to hold for the powder and concentrated solution dip methods (Tables I, V, and VI and Fig. 3). It is

TABLE I

EQUIVALENT CONCENTRATIONS OF INDOLEBUTYRIC ACID FOR ROOTING CUTTINGS BY THREE DIFFERENT METHODS

Name of plant	Time of year treated	Concentration of indolebutyric acid*			
		Mg./l. (24 hrs.)	Mg./cc. (dip)	Mg./g.** (dip)	Mg./g.† (dip)
Abies veitchii Murr. (fir)	Jan.	60–80	10–20	10–50	2–12
Acer palmatum Thunb. (Japanese maple)	May–June	10–40	—	—	2–5
Actinidia arguta Miq.	Dec.–Jan.	20–40	1–4	4–10	—
Begonia sp. var. Marjorie Gibbs	Dec.	1–5	—	—	1–2
Begonia semperflorens Link. & Otto	Nov.	1–5	—	—	1–2
Camellia japonica L. var. *chandleri elegans*	Jan.	60	4–10	25–50	—
Caragana boissii Lam.	May	10	4	—	2–5
Carya pecan Aschers & Graebn. (pecan)	Apr.	40	—	—	2–12
Catalpa sp.	Apr.	—	4	—	5–12
Celastrus articulatus Thunb. var. *punctatus* (bittersweet)	Nov.–Apr.	40	1–4	4–25	1–2
Cerastium sp.	Apr.	2–5	—	—	1–5
Chamaecyparis obtusa Sieb. & Zucc. var. *lutea nova*	Apr.	40–60	—	—	5–12
Chamaecyparis obtusa Sieb. & Zucc. var. *filiformis*	Apr.	40–60	—	—	5–12
Chamaecyparis pisifera Sieb. & Zucc. var. *plumosa aurea*	Apr.	40–60	—	—	5–12
Chamaecyparis pisifera Sieb. & Zucc. var. *filifera aurea*	Nov.	60	—	10–25	5–12
Chilopsis linearis D.C. (desert willow)	Jan.	60	4	10–25	—
Chrysanthemum sp.	Aug.–May	1–10	—	4–10	1–5
Crassula sp.	May	40	—	—	2
Cryptomeria japonica D. Don	Dec.	40–80	4–10	10–25	—
Dahlia variabilis Desf. (dahlia)	Apr.	20	4	—	2–5
Dianthus caryophyllus L. (carnation, English varieties)	Nov.–Mar.	5–10	—	4–10	1–5
Diervilla hybrida Dipp. var. Mme. Billard (weigela)	Oct.	5–10	4	—	1–2
Euonymus radicans Sieb.	Nov.–Apr.	5–10	1–4	—	1–5
Euphorbia pulcherrima Willd. (poinsettia)	Apr.	2–10	—	—	2
Gossypium hirsutum L. (cotton)	Mar.	—	—	—	5–12
Hedera helix L. (ivy)	Nov.	1–5	1	1–4	1–2
Heliotropium sp. (heliotrope)	Jan.–Feb.	10	—	—	1–2
Hibiscus syriacus L. (althea)	Oct.–Feb.	40–60	4–10	4–50	2–18
Ilex aquifolium L. (English holly)	May	20–40	—	—	5
Ilex opaca Ait. (American holly)	Nov.	40	4–10	10–50	—
Juniperus chinensis L.	Mar.	60	4	—	2–5
Juniperus chinensis L. var. *pfitzeriana* Spaeth.	Dec.–Jan.	60–80	4–10	10–25	2–12
Juniperus communis L. var. *montana* Ait.	Apr.	40–60	—	—	5–12
Juniperus conferta	Apr.	40	—	—	2–12
Juniperus virginiana L. var. *tripartitia* R. Smith	Apr.	40–60	—	—	5
Kolkwitzia amabilis Graebn.	June	20	—	—	12
Ligustrum ovalifolium Hassk. (privet)	Oct.–Nov.	40	4–10	10–50	—
Ligustrum ovalifolium Hassk. (privet)	May	80	10	—	2–12
Pachysandra terminalis Sieb. & Zucc.	Feb.	20–40	—	—	5–12
Picea abies (L.) Karst. (spruce)	Jan.–Mar.	20–40	—	—	2–5
Picea glauca Voss. var. *conica* (spruce)	Mar.	40–60	4	—	2–12
Prunus cerasifera Ehrh. var. *woodii*	May	—	4	—	2–5
Prunus persica Sieb. & Zucc. (Elberta peach)	Nov.	20–40	1–4	10–25	—
Prunus serrulata Lindl.	June	—	4	—	1–2

TABLE I—*Continued*

Name of plant	Time of year treated	Concentration of indolebutyric acid*			
		Mg./l. (24 hrs.)	Mg./cc. (dip)	Mg./g.** (dip)	Mg./g.† (dip)
Pyrus malus L. (Grimes Golden apple)	Nov.	40	4–10	10–25	—
Pyrus malus L. (Rhode Island Greening apple)	Nov.	40	4–10	10–25	—
Rhododendron sp. (*catawbiense* hybrids)	Dec.	40–80	10–20	10–50	5–12
Rhododendron sp. var. Caractacus	Apr.	—	10–20	—	5–12
Rosa sp. var. Briarcliff (rose)	Sept.–Mar.	1–2	0.3–1	—	1–2
Rosa sp. var. Crimson Rambler (rose)	May	1–5	1–4	—	1–2
Styrax americana Lam.	Dec.	—	4	25	—
Syringa emodi Wall. (lilac)	May	40	4	—	2–12
Syringa vulgaris L. (lilac)	Apr.–May	20–60	4–10	—	2–12
Taxus cuspidata Sieb. & Zucc. (yew)	Oct.–Feb.	60	4–10	10–50	2–5
Thuja occidentalis L. var. *ellwangeriana* Beissn.	Apr.	40	4	—	—
Thuja occidentalis L. var. *globosa nana*	Nov.–Apr.	20–60	4	—	2–12
Thuja occidentalis L. var. *robusta* Carr.	Nov.	60	4–10	25–50	5–12
Thuja occidentalis L. var. *spiralis* Hort.	Jan.–Apr.	60	4	4–25	2–12
Tsuga canadensis Carr. (hemlock)	Dec.–Feb.	40–60	4–20	10–50	5
Ulmus americana L. (elm)	Mar.	10–20	2–4	—	2–5
Vaccinium corymbosum L. var. Rubel (blueberry)	June–July	20–40	—	—	2–5
Viburnum carlesii Hemsl.	June	—	4	—	2
Vitis sp. (Concord grape)	Oct.	40–60	4–10	—	—
Vitis sp. (Concord grape)	Nov.	40	4–10	10–25	2–10

* Equivalent concentration values are described under "Materials and Methods."
** Commercial grades of talc and acid mixed in proportions indicated.
† Specially prepared finely ground mixtures of talc and indolebutyric acid.

believed that this relationship is a far more reliable criterion of the relative activity of different root-inducing substances than the percentage of cuttings rooted, since marked differences may be shown even when all cuttings (including controls) are rooted. For example, the marked difference in the rooting response illustrated for *Euonymus* in Figure 2 G would be entirely lost if the results were expressed in terms of the percentage of cuttings rooted. On the latter basis the poorly rooted dry controls and the 5 mg./g. lots which show 100 per cent rooting (Fig. 2 G, lower row) would be rated equivalent to the excellently rooted 5 mg./g. wet-treated lot appearing on the right in the upper row.

Concentration requirements varied not only with the species (Table I) but also with the relative age of the cutting with particular reference to the degree of hardness where the basal cut was made. Thus the optimum concentrations for succulent tip cuttings of chrysanthemum were 1 to 5 mg./l. and 0.5 to 1 mg./g., whereas for cuttings with harder stems the optimum concentrations were 5 to 10 mg./l. and 2 to 5 mg./g. (Table II). Both types of cuttings may be secured from the same plant or from shoots on plants grown under different conditions, as for example at different temperatures or at different times of the year. In contrast to chrysanthe-

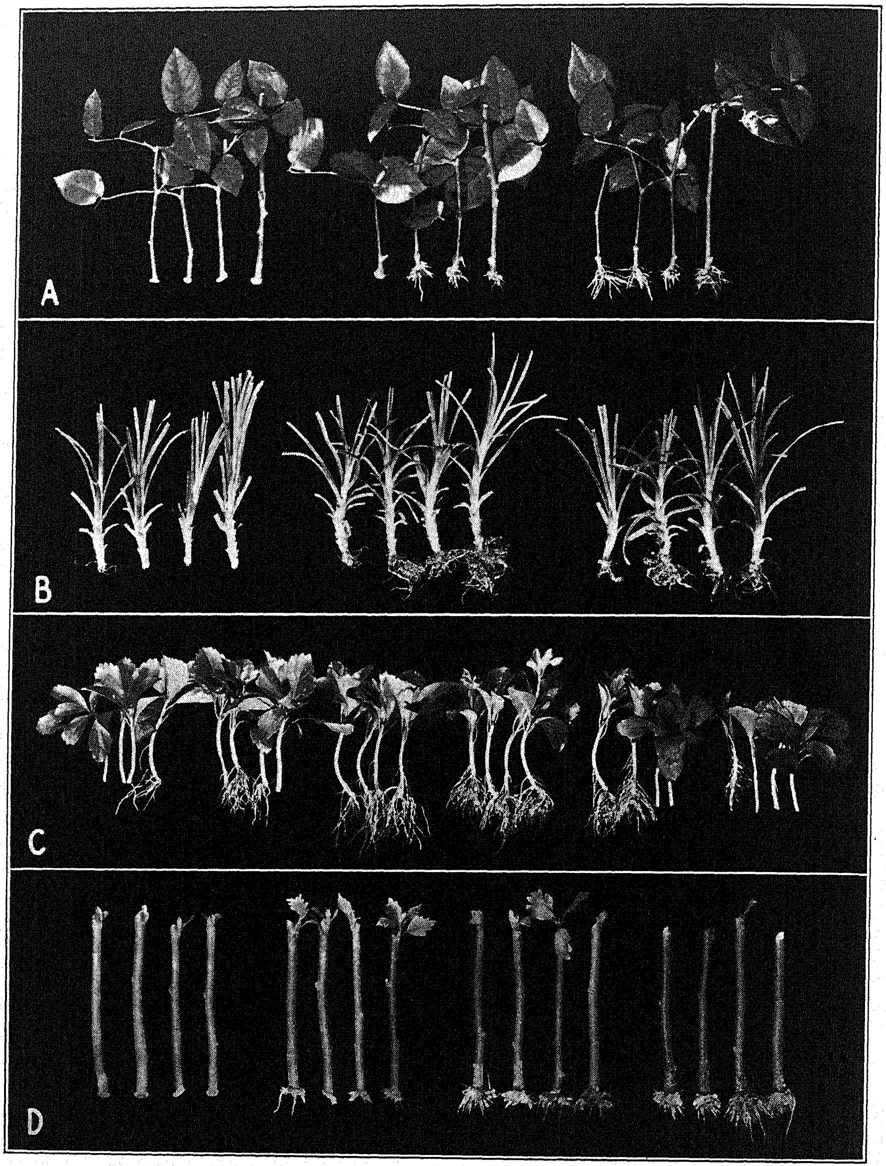

FIGURE 1. Concentration requirements (indolebutyric acid powder) for different species. A. Briarcliff rose (left to right): talc control, 1 and 2 mg./g. respectively. B. Carnation var. Pelargonium (left to right): talc control, 2 and 5 mg./g. respectively. C. *Pachysandra terminalis* (left to right): talc control, 2, 5, 12, 18, 35 mg./g. D. *Hibiscus syriacus* (left to right): talc control, 6, 18, 35 mg./g.

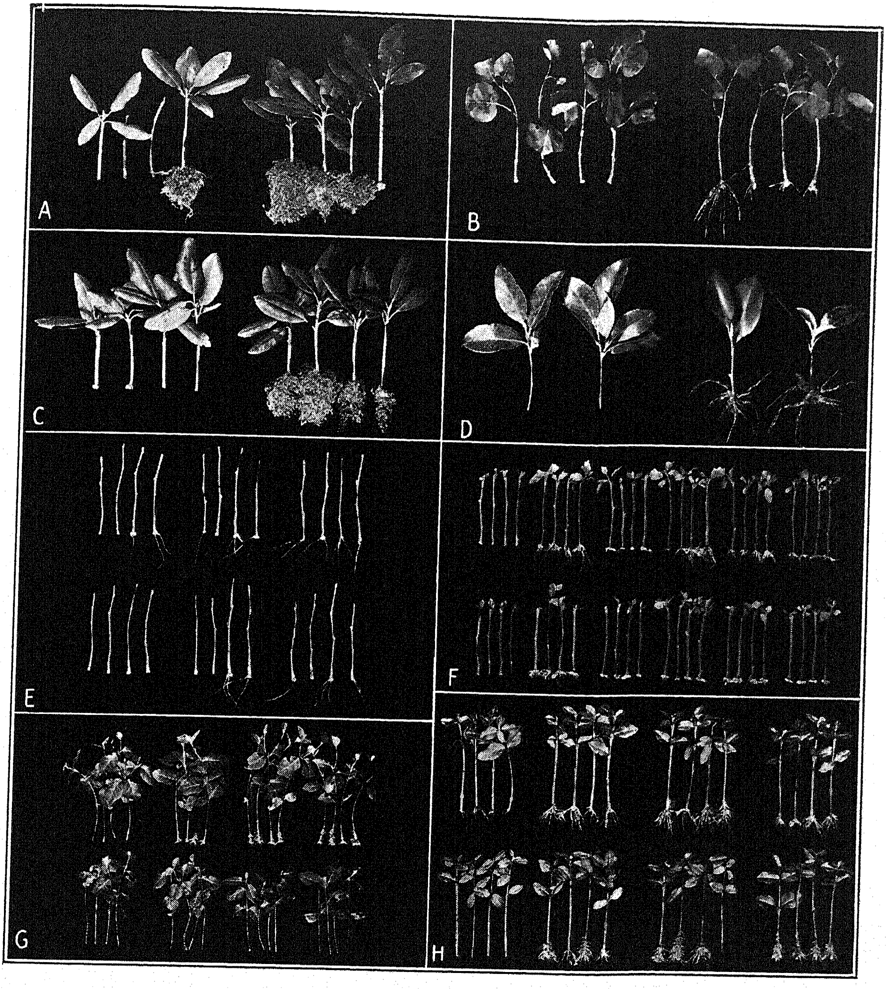

FIGURE 2. Response of cuttings to indolebutyric acid or the K-salt. Key: mg./g. signifies powder dip; mg./cc., solution dip; and mg./l., 24-hour immersion method. A. Rhododendron (left to right): check, 25 mg./g. acid. B. Lilac var. Arthur Wm. Paul (left to right): check, 12 mg./g. acid. C. Rhododendron (left to right): check, 10 mg./cc. K-salt. D. *Camellia japonica* var. *chandleri elegans* (left to right): two checks, 10 mg./cc., 25 mg./g. acid. E. Grimes Golden apple. (Upper) Acid solution (left to right): check, 4 and 10 mg./cc. respectively. (Lower) Acid powder (left to right): check, 10 and 25 mg./g. respectively. F. *Hibiscus syriacus.* (Upper) Bases washed at different intervals after treatment with 25 mg./g. acid. (Lower) Washed, then retreated. Left to right (both rows): check; treated but not washed; washed after 2 hours, 1 day, 3 days, 7 days respectively. G. *Euonymus radicans.* (Upper) Wet cuttings. (Lower) Dry cuttings. Left to right: check; 1, 2, 5 mg./g. acid respectively. H. *Euonymus radicans.* (Upper) Acid. (Lower) K-salt. Left to right (both rows): check; 4 and 10 mg./g.; 20 mg./l. (24 hours).

TABLE II

ROOTING RESPONSE OF CHRYSANTHEMUM CUTTINGS TREATED WITH INDOLEBUTYRIC ACID APPLIED IN DIFFERENT FORMS. AVERAGE NUMBER OF ROOTS PER CUTTING

Variety	Date treated	Date of root count	Concentration of indolebutyric acid								
			Mg./g. (dip)				4 mg. per cc. (dip)	Mg./l. (24 hrs.)			
			0*	1	2	5		0**	1.25	2.5	5
Rowena	Apr. 6	Apr. 17	4	—	9	—					
Valencia	Apr. 6	Apr. 17	10	49	42	47					
Melba	Apr. 6	Apr. 17	6	—	25	14		1	20	—	—
Heldaberrs	Apr. 6	Apr. 17	6	—	20	31					
Teton	Apr. 6	Apr. 17	6	—	20	30					
Harvard Red	Apr. 6	Apr. 17	2	6	14	13		1	—	5	—
Harvard Red	Apr. 14	Apr. 25	13	20	13	17	9	6	—	15	19
Peggy Ann Hoover	Apr. 6	Apr. 19	5	8	5	8		3	12	9	—
Peggy Ann Hoover	Apr. 14	Apr. 25	8	—	16	21		4	—	11	14
Peggy Ann Hoover	May 26	June 8	10	—	14†	—	8	3	—	—	10
Hilda Bergen	Apr. 14	Apr. 25	21	—	59	91					
Red Bird	Apr. 20	May 4	13	—	40	44		7	—	39	45
Red Bird	May 26	June 6	20	—	121†		47	11			140
Nugget	Apr. 26	May 9	8	—	23	25		13		18	25
Nugget	May 26	June 8	15	—	35†		30†	2	—	—	22
Yvalda	May 26	June 8	28	—	45†		0†	13	—	—	47

* Finely ground control talc.

** Tap water control, 24-hour treatment.

† Some or all cuttings definitely injured, as evidenced by excessive proliferation, discoloration, or killing of lower part of stem, or noticeable retardation of root growth.

mum, the tip cuttings of *Hibiscus syriacus* required higher concentrations than cuttings made from the remaining portion of the shoot. These differences are well-known and have been previously discussed in connection with solution treatments (4). The relative age of the cutting is thus an important limiting factor in determining the rooting response of cuttings treated with powders and with concentrated solutions.

The time of year treatments were administered also proved to be an important limiting factor in the response of cuttings to root-inducing substances. As previously reported for the named varieties of *Syringa*

TABLE III

REDUCTION IN NUMBER OF ROOTS CAUSED BY COATING BARK ON LOWER PART OF BRIARCLIFF ROSE CUTTINGS WITH PARAFFIN BEFORE TREATMENT WITH THE ROOT-INDUCING SUBSTANCE

Treatment with indolebutyric acid	Bark not coated	Bark coated*
24-hour treatment 2.5 mg./l.	39	17
Solution dip method 1 mg./cc.	38	13
Powder dip method 4 mg./g.**	11	2
Column totals	88	32

* Root-inducing substance in direct contact only with basal cut surface.

** Mixture not finely powdered.

vulgaris which responded to solution treatment only when taken the latter part of April or during May (6), the same results were obtained during 1939 with the powder treatments. Even though young shoots of the lilac taken during May are difficult to maintain in good condition in the rooting medium, they tolerate and require relatively high concentrations of indolebutyric acid (40 to 60 mg./l., 20 mg./cc., and 5 to 12 mg./g.). In addition to the 11 varieties tested during 1938 and for which 50 to 100 per cent rooting was obtained in 40 to 60 days, two other varieties (Reine Elizabeth and Siebold) gave 100 per cent rooting in 25 to 28 days when treated with indolebutyric acid in a concentration of 12 mg./g. (Table IV). These results indicate that the named varieties of *Syringa vulgaris*, generally referred to as French lilacs, can be rooted readily and there should be no necessity in the future for grafting them. Rooted cuttings from the 1938 tests made an excellent growth in the field during the spring of 1939. Re-

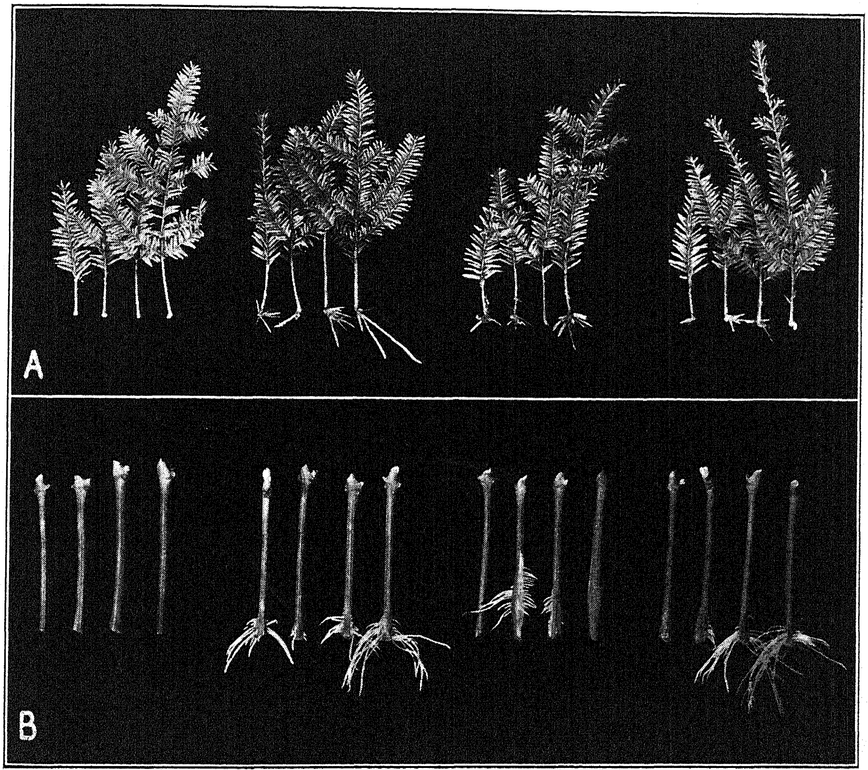

FIGURE 3. Comparative rooting for indolebutyric acid applied according to different methods to (A) *Tsuga canadensis* and (B) Concord grape. Left to right (A and B): nontreated control, 40 mg./l. (24 hours), 4 mg./cc. in 50 per cent alcohol (dip method), and 5 mg./g. powder (dip method).

sults with lilac are similar to those obtained with *Taxus cuspidata* and *Tsuga canadensis* for cuttings treated in June. Control cuttings of *T. canadensis* were previously rooted only when taken from June to August (2). However, seasons vary from year to year so that specific dates cannot be considered as an accurate indication of the relative age of the shoot.

TABLE IV

ROOTING OF LILAC (SYRINGA VULGARIS) TREATED WITH INDOLEBUTYRIC ACID APPLIED AS A POWDER. AVERAGE NUMBER OF ROOTS PER CUTTING

Variety	Day treated (May)	Root counts made (June)	Total No. days	Conc. of acid in mg. per gram talc			
				0*	2	5	12**
Arthur Wm. Paul	19	12	24	0	9	3	23
Clara Cochet	19	23	35	0	2	0	4
Perle von Teltow	19	23	35	0	0	0	9
Dame Blanche	15	23	39	0.3	1	1	7
Siebold	18	14	27	0	3	2	13
Toussaint Louverture	18	16	29	0	2	1	6
Reine Elizabeth	19	13	25	0	7	3	18
Reine Elizabeth	19	16	28	0	0.3	2	12
Prof. Sargent	19	16	28	0.3	4	4	21
Prof. Sargent	15	23	39	0	3	1	14
Mme. Florent Stepman	10	16	37	0	1	8	0
Mme. Florent Stepman	15	23	39	0	2	0	4
Column totals				0.6	34.3	26	131

* Finely ground control talc corresponding to that used in mixtures of the acid and talc.

** 100 per cent rooting obtained with this concentration except in the case of Mme. Florent Stepman.

Cuttings of many species of plants required higher concentrations of indolebutyric acid when taken during October and November as compared with other times of the year. The following species or varieties exhibited the differences mentioned: *Taxus cuspidata, Thuja occidentalis* varieties *globosa nana, robusta, spiralis*, and *ellwangeriana, Juniperus chinensis* var. *pfitzeriana, Chamaecyparis obtusa* varieties *filiformis* and *filifera aurea, Tsuga canadensis, Abies veitchii, Hibiscus syriacus*, Concord grape, *Ligustrum ovalifolium, catawbiense* hybrid rhododendrons, and *Celastrus articulatus* var. *punctatus*. Considering these results and others described in this section, it appears that regardless of the method of application a given concentration of a root-inducing substance does not produce optimum rooting for all species and not even for the same species at all times of the year and under all conditions normally met with in propagation procedures. In general, cuttings of evergreens have shown more variation than cuttings of deciduous plants. For this reason the approximate optimum concentrations for the two dip methods listed in Table I cover a wider range than in the case of evergreens treated according to the standard immersion method. Strict agreement in the results of different workers cannot be expected unless the principal factors which determine concentration requirements for a given root-inducing substance have been accounted for. The use of different root-inducing substances introduces additional factors which the results reported in other sections of this paper will show may be of a more limiting nature than those discussed in this section for indolebutyric acid.

TABLE V

COMPARATIVE RESULTS WITH ACIDS AND SALTS APPLIED BY DIFFERENT METHODS TO CUTTINGS OF EUONYMUS RADICANS. AVERAGE NUMBER OF ROOTS PER CUTTING

Treatment	Conc., mg. per unit of carrier	Indolebutyric		Naphthaleneacetic	
		Acid*	K-salt**	Acid*	K-salt**
Standard method 24-hr. treatment mg./l.	20 0	21 1	56 —	8 —	11 —
Solution dip method mg./cc.	10 4 0	38 51 3	59 44 1	20 10 —	13 6 —
Powder dip method mg./g.	10 4 0	25 14 5	37 31 —	12 8 —	32 11 —
Column totals	—	158	228	58	73

* Dissolved in 50 per cent ethyl alcohol.
** Dissolved in water.

The effect of bottom heat (Table VI) shows that rooting of *Hibiscus* cuttings varied according to the temperatures used. Carnation responded in a manner similar to *Hibiscus*. In general, any beneficial effects of bottom heat (78° to 80° F.) consisted mainly of quicker rooting and a more rapid rate of root growth. Many species rooted better at 70° to 73° F. than at 78° to 80° F. These latter results were due primarily to the poor condition of the cuttings held at the higher temperature. No attempt was made in these tests to determine optimum temperatures for air and for the medium. The special case reported is used to illustrate the fact that a favorable temperature of the medium may act in effect the same as a higher concentration and hence temperature is a limiting factor when determining minimum concentration requirements for rooting of cuttings.

Optimum concentrations (75 to 100 per cent rooting) of indolebutyric acid powder for species not appearing in the tables are as follows: *Abelia grandiflora* Rehd. var. *rosea alba* (June 15) 2 mg./g.; *Chrysanthemum coccineum* Willd. (June 9) 2 mg./g.; *Cornus florida* L. (June 1) 5 to 12 mg./g.; *Corylus avellana* L. (June 16) 5 mg./g.; *Euphorbia pulcherrima* Willd. (May 1) 1 to 2 mg./g.; *Juniperus chinensis* L. var. *japonica* Vilm. (March 1) 5 mg./g.; *Lagerstroemia indica* L. (June 20) 2 mg./g.; *Lonicera japonica* Thunb. (April 14) 2 mg./g.; *Philadelphus pubescens* Loisel. var.

pendulifolius (May 16) 1 mg./g.; *Picea abies* Karst. (March 1) varieties *cupressina* 5 mg./g. and *echinaeformis* 2 mg./g.; *Prunus tomentosa* Thunb. (June 15) 2 mg./g.; *Pyrus serotina* Rehd. (June 1) 2 mg./g.; *Rhododendron mucronatum* Don. (May 31) 2 mg./g.; *Sophora viciifolia* Hance (June 15) 2 mg./g.; *Syringa vulgaris* L. varieties Mont Blanc (May 10) 2 mg./g., Oliver de Serres (May 18) and Ronsard (June 1) 12 mg./g.; *Tilia platyphyllos* Scop. (June 1) 2 mg./g.; *Vitex agnus-castus* L. (June 27) 5 mg./g. The date of treatment was specified in these tests (1939) since the time of year cuttings are taken is critical for some species, but the equivalent time may vary from year to year. For example, shoots of *Syringa vulgaris* varieties taken between the 10th and 15th of May, 1939, were comparable in age and relative activity with those taken between April 28th and May 2nd, 1938. Shoot growth was correspondingly delayed on other species of plants during the spring of 1939.

TABLE VI

INFLUENCE OF BOTTOM HEAT ON ROOT FORMATION IN CUTTINGS OF HIBISCUS SYRIACUS TREATED* WITH INDOLEBUTYRIC ACID BY THREE METHODS. AVERAGE NUMBER OF ROOTS PER CUTTING

	Standard method, mg./l.					Powder dip method, mg./g.**					Solution dip method, mg./cc.			
	0	20	40	60	Group totals	0	6	18	35	Group totals	0	4	10	Group totals
Bottom heat 78° to 80° F.	0	4	4	11	19	3	16	26	53	98	0	19	34	53
Control bed 70° to 73° F.	0	2	3	6	11	2	7	5	1	15	0	6	9	15
Column totals	0	6	7	17	30	5	23	31	54	113	0	25	43	68

* Treated January 20 and data recorded 17 days later.

** Mixtures and control talc finely powdered.

Since control cuttings treated with talc showed consistently a slightly better rooting than non-treated controls or controls immersed in tap water, the effect of talc itself must be accounted for in experiments relating to results with comparative methods. In the case of young tip cuttings of chrysanthemum (Table II), talc controls remained in good condition whereas non-treated controls wilted each day for a period of several days and in some cases for the period of the test. Young carnation cuttings responded in a similar manner. This effect of talc appears to be due at least in part to differences which involve water relations. However, in many species there was no evidence of wilting but the talc controls showed better rooting and better callus formation than non-treated controls or tap water controls. The effectiveness of talc itself has also been observed by Stoutemyer (7). He reports percentage rooting values for cuttings treated with talc which represent in some cases much greater increases over the non-talc controls than any treatment with root-inducing substances over the talc controls (7, p. 818–819).

Results of some recent experiments indicate that the activity of control talc (Merck & Co. Inc.) may be due in part to the presence of a physiologically active ingredient which was obtained by extracting talc with chloroform. The chloroform residue was active when tested on tomato plants. This work is being continued for the purpose of determining the identity and the quantities of a physiologically active substance in different grades of talc and in other powders which might prove suitable as carriers for growth substances.

RELATIVE ACTIVITY OF DIFFERENT SUBSTANCES

There is general agreement in published reports as previously pointed out (4) that indolebutyric and naphthaleneacetic acids are more effective for rooting cuttings than indoleacetic acid, and that indolebutyric acid is the most effective of the three, from all standpoints, for practical use. Results with the concentrated solution and powder dip methods confirmed the results obtained with the standard immersion method of treatment in showing that indolebutyric acid was more effective on cuttings of most species than either naphthaleneacetic or indoleacetic acids. These experiments included the use of species requiring relatively low, medium, and high concentrations of the root-inducing substances (chrysanthemum, rose, *Euonymus*, *Hibiscus*, and California privet). Exceptions to the general rule just mentioned are known and have been reported (9, 4). For example, the fact that naphthaleneacetic acid is much more effective on privet than either indoleacetic or indolebutyric acids has been verified by tests in which the substances were applied as dilute solutions (5 to 80 mg./l.), as concentrated solutions (1 to 20 mg./cc.), as powders (1 to 12 mg./g.), and as vapors (10), the concentrations of which were not known. In a similar manner tests with all four methods showed that *Euonymus radicans* responded best to indolebutyric acid.

Whereas the optimum range of concentrations of indolebutyric acid is 1 to 80 mg./l. for the species listed in Table I, the optimum range of concentrations of indoleacetic acid for many of these same species is considerably higher as previously reported (4). Thimann and Delisle give 25 to 400 mg./l. as the optimum range of indoleacetic acid for all species which they tested and 100 to 400 mg./l. as the range for species difficult to root (8). Hubert *et al.* (5) found 100 to 150 mg./l. to be the optimum range for indoleacetic acid for species difficult to root. *Tsuga canadensis*, which is difficult to root (except during June and July when many controls have rooted), requires a concentration of indolebutyric acid of 40 to 60 mg./l. (Table I and Fig. 3 A). These results are to be contrasted with those reported by Thimann and Delisle, who found it necessary to use indoleacetic acid in concentrations of 400 mg./l. to obtain equivalent (100 per cent) rooting of *Tsuga canadensis* cuttings (8). These results indicate that indolebutyric acid is about ten times more effective than indoleacetic acid for cuttings of *Tsuga canadensis*. Although cuttings of *Tsuga canadensis* taken during June root more readily than at other times of the year, they are difficult to maintain in good condition just as is described for the May shoots of lilac. In both cases the shoots had not attained their full length or leaf size. Treatment of cuttings with indolebutyric acid has not shown the age of the parent plant to be an important limiting factor, and certainly not the principal limiting factor, as claimed by Thimann and Delisle in the case of treatments with indoleacetic acid (8). Cuttings of *Tsuga canadensis* and *Picea pungens* taken from large trees (10 to 30 years old) rooted readily without the use of root-inducing substances when taken at the proper time of the year (2).

Potassium salts of indolebutyric, indoleacetic, and naphthaleneacetic acids were generally more effective than the acids as illustrated in Figure 2 H and Tables V and VII. The higher effectiveness of the salts for root formation was particularly noticeable in the case of the two dip methods. In most cases the tests constituted a comparison between the acid and salt of one or two substances. However, in one test the three principal acids and their corresponding potassium salts were compared simultaneously in concentrations of 2, 5, and 12 mg./g. on cuttings of chrysanthemum, rose, *Euonymus*, and privet. Although previously the salts were reported to be about equally as effective as the acids in the case of the standard immersion method of treatment (9, 4), the results obtained with the dip methods indicate that the salts are sufficiently more active than the acids to be of practical value in propagation. The potassium salts were more active at all three concentrations than the acids. In the case of the 12 mg./g. powder, the salt of naphthaleneacetic acid caused toxic and over-treatment effects on privet cuttings characteristic of an excessively high concentration. These effects consisted of excessive swelling and proliferation of the stems, the formation of many short roots which were inhibited in growth, and the distortion of the terminal growth. The newly formed leaves were small, savoyed, and exhibited downward rolling at the margins. Similar abnormalities and toxic effects were not caused by lower concentrations of the salt or by the same concentration (12 mg./g.) of the acid.

The high effectiveness of the concentrated solutions (1 to 20 mg./cc.), particularly for species relatively difficult to root, makes this method worthy of further consideration. Cuttings which responded favorably to the concentrated solutions of the acid and the K-salt of indolebutyric acid were rhododendron hybrids, *Tsuga canadensis*, *Abies veitchii*, *Ilex opaca*, *Ulmus americana*, *Picea glauca* var. *conica*, several varieties of *Thuja occidentalis* and *Chamaecyparis obtusa*, *Juniperus* sp., *Camellia japonica* varieties *alba plena* and *chandleri elegans*, varieties of *Syringa vulgaris*, and certain varieties of the commercial fruiting apple. The effectiveness of the potassium salt of indolebutyric acid on hybrid rhododendron cuttings is of particular interest and indicates that this and other salts might prove of considerable practical importance.

TABLE VII

Relative Activity of Acids and Salts for Inducing Root Formation in Hardwood Leafless Cuttings of Hibiscus syriacus*

Form of substance	Indolebutyric, mg./g. or mg./cc.					Naphthaleneacetic, mg./g. or mg./cc.				
	0	4	10	20	Totals	0	4	10	20	Totals
A. Powder dip method**										
Acid	0	5	9	—	14	0	2	5	—	7
K-salt	0	8	19	—	27	0	3	7	—	10
B. Solution dip method										
Acid	0	18	17	28	63	0	6	10	2	18
K-salt	0	22	21	42	85	0	3	4	15	22

* Treated November 11 and data recorded December 19, 1938.
** Control talc and mixtures not finely powdered.

FACTORS INFLUENCING SOLUBILITY AND PENETRATION

Treating dry cuttings with powders as recommended by Grace (1) proved relatively ineffective on most species. Wetting the basal one-half inch of the cuttings with water just before dipping into the powders proved much more effective (Fig. 2 G and Table VIII). Wetting the cuttings with 50 to 95 per cent ethyl alcohol proved more effective than wetting with water. Since the acids are more soluble in alcohol than in water, these results indicate that the presence of a solvent on the treated surface of the stem increases solubility of the crystalline root-inducing substance. It was

TABLE VIII

Effect of Wetting Cuttings of Taxus cuspidata before Treatment with Talc Preparations of Root-Inducing Substances. Average number of Roots per Cutting after Two Months*

Condition of basal ends when treated	Indolebutyric acid in mg./g. talc**					Totals	Naphthaleneacetic acid in mg./g. talc					Totals
	0	1	4	10	50		0†	1	4	10	50	
Dry	3	1	2	1	1	8	1	1	4	4	6	16
Wet	4	3	4	8	17	36	1	7	9	21	27	65

* Treated October 19, 1938.
** Control talc and mixtures not finely powdered.
† Normal controls.

found that more of a given powder (about three times by weight) adhered to the wet cuttings than to the dry cuttings. However, the fact that salts were more effective than the acids, and that wetting the cuttings with alcohol was more effective than wetting with water, indicates that solubility and possibly penetration were more important than the additional amounts of powder adhering to the surface of the cutting. The higher activity of the salts as compared with the acids is at least partly explainable on the basis of the higher solubility of the salts in water.

The question of whether the powder treatments are effective because they supply the cuttings with just the proper quantities of the root-inducing substance over a period of several days or weeks, appears to be answered in the negative by results of tests in which the powder was washed off at various intervals after being applied. Data for hardwood leafless cuttings of *Hibiscus* (Table IX) show that if the powder remains on the cutting for as short a period as two hours there was sufficient penetration to induce much better rooting than on control cuttings. There was relatively little difference in the rooting of cuttings washed one, three, and seven days respectively after application of the powder. A similar series of cuttings retreated after washing off the powder (Table IX and Fig. 2 F) showed a slightly increased rooting response with respect to the number of roots induced, but in all of the retreated lots the roots were shorter than in the case of the lot not washed and not retreated. Control lots had not rooted in this time. These results indicate that the principal action of the powder occurs within a relatively short time and probably in many cases within the first 24 hours. With respect to number of roots and length of roots (Table IX), retreatment appeared to act in effect the same as a higher concentration. In these tests all of the cuttings were planted after receiving the initial treatment and then they were removed for retreatment and

TABLE IX

EFFECT ON ROOT FORMATION OF WASHING BASAL ENDS OF TREATED HIBISCUS CUTTINGS AT DIFFERENT INTERVALS AFTER THE TALC PREPARATION HAD BEEN APPLIED*

Treatment	Average number of roots						Average length of roots (mm.)				
	Non-treated controls	Treated, not washed	Time cuttings were washed (after)				Treated, not washed	Time cuttings were washed (after)			
			2 hrs.	1 day	3 days	7 days		2 hrs.	1 day	3 days	7 days
Washed	0	25	19	33	31	31	31	6	26	15	16
Washed, retreated	0	32	26	38	58	49	18	6	7	10	6

* 25 mg. indolebutyric acid per gram of talc (control talc and mixtures not finely powdered).

planted as originally. The process of removing and replanting the cuttings may have been a factor in causing some of the differences described.

Data in Table III indicate that there is considerable penetration of the root-inducing substance through the bark of Briarcliff rose cuttings since covering the bark with paraffin before treatment with the powders reduced the number of roots which emerged. These results are in agreement with those previously reported for solution treatments (4). In both tests effective concentrations caused roots to emerge from tissue where roots do not normally emerge in control cuttings, even from tissue located several inches above the region where the substance was originally applied. Results with root-inducing substances applied as dilute or concentrated solutions, as powders, and as vapors all show that in the case of species responding to treatment roots emerge from stem regions characteristic for the species and also from other regions, including all levels from base to tip, depending upon the species and the concentration of the substance.

DISCUSSION

Tests with root-inducing substances show that optimum rooting varies with the species, the age and relative activity of the shoot, the time of year treatment is administered, the kind and concentration of substance, and the method of applying the substance to the cutting. In the case of all methods the concentration of root-inducing substance varied with the species tested. These results are in agreement with those reported extensively in the literature with particular reference to solution treatments. However, relatively few reports deal with the use of powder preparations. In the present tests the results show that the use of powder preparations of root-inducing substances involves essentially the same factors which have proved limiting in the case of solution treatments. The principal differences were quantitative.

With respect to concentration requirements on a weight basis the concentrated solutions (1 to 20 mg./cc.) and powder preparations (1 to 12 mg./g.) were about equally effective for a given species, but they represent values 10 to 1,000 times higher than equivalent concentrations (1 to 80 mg./l.) used for the standard immersion method of treatment. While young shoots of woody and herbaceous plants generally responded more readily than older shoots to lower concentrations of root-inducing substances, it was observed that young shoots of some species (lilac, privet, Japanese maple, hemlock) tolerated and required relatively high concentrations for optimum rooting.

Lilac cuttings responded only slightly or not at all to treatment with 2 to 5 mg./g. but they responded exceptionally well when treated with 12 mg./g. Data for lilac show that a proportional increase in rooting did not occur as a result of an increase in concentration from 2 mg./g. to 5 mg./g. (Column totals, Table IV), which is the lower part of the effective range. This is to be contrasted with the marked increase occurring with the use of 12 mg./g. which represents the higher part of the effective range. Since these results were typical of most other species difficult to root (rhododendron hybrids, English varieties of carnation, and certain evergreens), it is important when determining concentration requirements to include the entire effective range of concentrations. As representative of species difficult to root, the varieties of *Syringa vulgaris* are well suited for root formation tests. Tests covering a period of three years show conclusively that the nine varieties reported in the present paper and seven other varieties reported by Kirkpatrick (6) can be rooted successfully (50 to 100 per cent) when treated with indolebutyric acid applied as a solution or a powder and thus need not be grafted as has been the common practice in the past. Rooted cuttings of these varieties have shown a vigorous growth when planted in the field.

The age of the shoot proved to be an important limiting factor in the case of all methods of treatment. Likewise, the time of year cuttings were treated was an important factor. Not all the differences are explainable solely on the basis of the degree of maturity or on the basis of the relative hardness of the stem. Whereas the lilac rooted readily only from the young shoots taken in May, the Concord grape, *Hibiscus*, and many evergreens rooted more readily before and after the months of October and November. At least in some species the degree of dormancy of the buds appears to influence rooting. The age of the parent plant was not an important limiting factor in tests with woody species since all plants were more than five years of age and in most cases more than ten years old.

Indolebutyric acid was more effective than naphthaleneacetic acid or indoleacetic acid on most species. In our tests this held true for the same species regardless of the method used for applying the root-inducing substance. The higher activity of the salts as compared to the acids, for the rooting of both softwood and hardwood cuttings, indicates that solubility and penetration are important factors regardless of how the root-inducing substance is applied. Grace (1) also found the K-salt more effective than the acid. Results relating to the use of different solvents, removal of the test powder (by washing) at various intervals up to several days after treatment, and coating the bark with paraffin all show that treatments which tend to increase solubility or penetration were most effective for rooting. Since the principal effect of the powder treatment occurred within 24 hours or less, adherence of the powder to the treated surface of the cutting appears not to be of any considerable importance after the first day. According to our results there should be no necessity for planting cuttings with greater care in the case of powder treatments than with other methods. Any powder which may be dislodged when the cuttings are planted appears to be of little importance if the proper concentration of an active root-inducing substance is applied initially.

Control cuttings treated with talc produced more roots per cutting and in a shorter time, and produced a greater quantity of callus on some species as compared with either non-treated controls or tap water controls. These differences were consistent but not pronounced in comparison with responses to treatment with talc preparations containing the root-inducing substance. At least part of the effect of talc appeared to involve water relations since the talc controls exhibited less evidence of wilting at all times and the bases of the cuttings showed less drying and discoloration as compared with non-treated or tap water controls. A similar effect in reducing wilting has been observed in tests in which the roots of tomato seedlings were dipped into colloidal clay (a finely ground clay containing phosphates and used commercially as a fertilizer) at the time of transplanting from flats. In addition to its influence on water relations, control talc was found to contain a physiologically active ingredient which was extracted with chloroform. The results with control talc indicate that it is of considerable importance in accounting for any beneficial effects of the carrier, particularly in experiments relating to the use of powder or dust preparations of root-inducing substances. Talc induced twice as many roots on chrysanthemum cuttings as compared with the number of roots on non-treated or tap water controls.

The higher activity of powder preparations which were furnished in a finely ground state as compared with similar preparations prepared by mixing commercial grades of talc and the root-inducing substances (Table I) is a point of considerable importance not only in experimental work but also in practice. Thus the concentrations specified for optimum rooting of a given species will depend not only upon the kind of active root-inducing substance, but also upon the physical characteristics of the powder. Until some standard type of powder is used, it seems likely that there will not be entire agreement in the results of different workers. While our results agree in the main with those of Stoutemyer (7) there are certain differences with respect to effective concentrations which may be due in part to the differences in the physical characteristic of the test powders.

SUMMARY

Treatment of cuttings with root-inducing substances applied as relatively dilute solutions (1 to 80 mg./l.), as concentrated solutions (1 to 20 mg./cc.), and as powders (0.5 to 50 mg./g.) produced essentially the same rooting response. Concentration requirements for optimum rooting varied according to the kind and form of substance, the kind of carrier or solvent, the species of plant, the age and relative activity of the shoot, the time of year treatment was administered, and the method of applying the substance to the cuttings.

The physical characteristics of the test powders appeared to account mainly for the differences in the effective range in concentration of 4 to 50

mg./g. for the coarser test powder and 0.5 to 12 mg./g. for the finer test powder. The concentrated solution (1 to 20 mg./cc.) and powder (0.5 to 12 mg./g.) dip methods were about equally effective on a weight basis but represent concentrations 10 to 1,000 times those producing equivalent rooting according to the standard solution method of treatment (1 to 80 mg./l.).

Potassium salts were consistently more effective than the acids (indoleacetic, indolebutyric, and naphthaleneacetic) which appeared to be due in part to solubility relations. Indolebutyric acid or the salt was more effective on most species than indoleacetic or naphthaleneacetic acids. The high activity of potassium indolebutyrate suggests that it may be of considerable practical importance.

Talc controls exhibited better rooting than non-treated controls or tap water controls. At least part of the beneficial effect of talc appeared to involve water relations since at all times the cuttings showed less evidence of lack of water than did the non-treated or tap water controls. An ingredient of control talc, soluble in chloroform, was found to be physiologically active when tested on tomato plants.

Treatment of some 70 species included types readily rooted, moderately difficult, and those definitely difficult to root. Varieties of *Syringa vulgaris* rooted in 24 to 39 days (50 to 100%) when treated with indolebutyric acid applied as a powder.

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EXPERIMENTS WITH VAPORS AND SOLUTIONS OF GROWTH SUBSTANCES

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Two recent publications (15, 16) concerned responses of plants induced with growth substance vapors in contrast to solutions. Twenty-nine acids and esters were found to be physiologically active when applied in the vapor form. The responses induced by vapors of these compounds were similar in most respects to those induced by the same substances in solution. Special interest has arisen in the new method of applying growth substances since the responses thus induced closely resemble those caused by exposure of plants to carbon monoxide and the unsaturated hydrocarbon gases (11, 12).

The purpose of the present paper is to report the results of additional experiments with vapors and solutions of growth substances and to list for the first time several new substances.

MATERIAL AND METHODS

Plants were exposed to vapors of growth substances under bell jars or large beakers. The chemicals were placed in small watch glasses and enclosed with the plants. The quantity required was not definitely determined. The equivalent of one drop or less of either crystalline or liquid substances was sufficient to make several exposures. The dosage was varied by length of exposure and by using the substances cold or warm. When heat was applied, as was necessary with the less volatile compounds, the watch glass containing the chemical was placed upon a hot crucible turned upside down under the bell jar. The length of exposure was varied from 10 seconds to 72 hours, depending upon the volatility of the chemicals and the type of plant material to be treated. Considerable experience was necessary to learn how to regulate the vapors to induce the optimum or maximum responses. The methyl and ethyl esters of naphthaleneacetic, indoleacetic, and indolebutyric acids were among the easiest to apply. Those placed upon a hot crucible for 30 seconds to 5 minutes induced pronounced responses. The esters of phenylacetic acids worked best without heat and with one or more hours' exposure. Leafless stems, tubers, corms, and the like were exposed longer than leafy cuttings or growing plants.

In this report, the capacity of a substance to induce epinasty of tomato leaves is taken as a criterion of unusual physiological activity and such chemical compounds are classified as growth substances. The active chemicals used in the present experiments are as follows:

Growth substances previously known to be active: α-naphthaleneacetic acid, methyl α-naphthaleneacetate, ethyl α-naphthaleneacetate, acenaphthyl-5-acetic acid, α-naphthylacetonitrile, 1-naphthaleneglycollic acid, 1-naphthaleneglyoxalic acid, α-naphthoxyacetic acid, β-naphthoxyacetic acid, β-naphthyl mercaptoacetic acid, β-naphthyl glycine, α-naphthoylpropionic acid, β-indoleacetic acid, methyl β-indoleacetate, ethyl β-indoleacetate, β-indolepropionic acid, methyl β-indolepropionate, β-indolebutyric acid, methyl β-indolebutyrate, ethyl β-indolebutyrate, indole α-methyl β-acetic acid, phenylacetic acid, methyl phenylacetate, ethyl phenylacetate, mandelic acid, *cis* cinnamic acid, and irradiated methyl cinnamate and ethyl cinnamate.

Substances active as vapors and now being reported for first time: β-naphthoxyacetamid, ethyl β-naphthoxy α-butyrate, β-naphthoxy α-butyric acid, ethyl α-naphthoxy α-butyrate, methyl and ethyl β-naphthoxyacetate, methyl and ethyl α-naphthoxyacetate, α-naphthoflavone, α-naphthylamine, α-naphthaleneacetic acid picrate, β-naphthoxyacetic acid picrate, α-naphthoxyacetic acid picrate, iso-butyl phenylacetate, butyl phenylacetate, methyl ethyl phenylethylmalonate, phenylbutyric acid, phenylethylacetic acid, *N*-phenylglycine ethyl ester, irradiated methyl *m*-nitrocinnamate, methyl α-trimethylamino-β-(3-indole) propionate iodide, ethyl α-bromoacetoacetate, homopiperonilic acid, nicotinic acid nitrate, pimelic acid, and *m*-tolyl β-naphthoxyacetate.

The naphthalene compounds, except naphthaleneacetic acid (Merck), naphthoflavone and naphthylamine (Eastman), were synthesized in the Institute laboratories. The indole compounds and homopiperonilic acid were supplied by Merck & Co. Inc. The others were purchased from Eastman Kodak Co.

Plant materials consisted of actively growing potted plants, plants with flower buds, leafy and hardwood cuttings, seeds, and tubers. The species are as follows: *Ardisia crispa* A.DC., *Bidens* sp. (Spanish needle), *Capsicum frutescens* L. (pepper), *Celastrus articulatus* Thunb. (bittersweet), *Cissus sicyoides* L. var. *jacquini* Planch. (tropical grape), *Cosmos sulphureus* Cav., *Epidendrum* sp. var. O'Brienianum (hybrid orchid), *Euonymus radicans* Sieb., *Fragaria* sp. var. Dunlap (strawberry), *Fuchsia hybrida* Voss. (fuchsia), *Heliotropium peruvianum* L. (heliotrope), *Hibiscus syriacus* L. (althea), *Hydrangea opuloides* Koch., *Ilex opaca* Ait. (American holly), *Kalanchoe daigremontiana*, *Laburnocytisus adamii* (golden chain), *Ligustrum ovalifolium* Hassk. (privet), *Lonicera* sp. (honeysuckle), *Lycopersicon esculentum* Mill. (tomato), *Medicago sativa* L. (alfalfa), *Mimosa pudica* L. (sensitive plant), *Nicotiana tabacum* L. var. Turkish (tobacco), *Petunia hybrida* Vilm., *Phlox paniculata* L., *Pisum sativum* L. (pea), *Rosa* sp. (hybrid tea rose and Paul's Scarlet), *Saxifraga sarmentosa* L., *Solanum melongena* L. var. *esculentum* Nees. (eggplant), *Solanum* *tuberosum* L. (potato), *Staphylea trifolia* L. (bladder-nut), *Syringa vulgaris* L. (lilac), *Vicia faba* L. (Windsor bean), and *Zea mays* L. (corn).

The tomato plant was used as a test object for vapors and solutions of all of the chemicals listed. The other species were used principally with vapors of indolebutyric, indoleacetic, phenylacetic, naphthaleneacetic, and naphthoxyacetic acids and the methyl and ethyl esters of these acids.

RESULTS

The responses induced with growth substances concerned in this paper are of a formative nature usually affecting particular organs or parts of a plant. In this respect they differ from fertilizers which promote the general growth of plants. When the substances are applied as vapors the entire plant is affected and though growth occurs the various tissues are not equally susceptible, resulting in abnormal shape of organs and direction of growth. Downward growth of leaves, enlarged stem tips, hypertrophies, and positive geotropism of stems are good examples of formative effects which might be referred to as induced abnormalities. Associated with these evident peculiarities are abnormal rates of metabolism involving a chain of responses. These will be discussed under special headings.

Though there are many qualitative differences in the responses induced by the 54 substances used in these experiments, they have in common the capacity to induce epinasty of tomato leaves. Even here there are differences in the type of induced epinasty and the degree of response. However, for the present we have elected to call "growth substances" all chemical compounds which have the capacity to cause epinasty of leaves (Fig. 1, A and C) similar to that induced by ethylene gas.

Other effects which most growth substances have in common are the capacity to initiate adventitious roots and retard the growth of active roots. There are, however, many qualitative differences in this connection.

This report concerns only a few of the more striking responses which can be induced with vapors of growth substances and some of the qualitative differences between the 54 physiologically active compounds listed under "Material and Methods." Plants also vary in their capacity to respond to a given substance. Many variations are shown in Table I.

First responses shown by motion pictures. Using leaf epinasty as an early indication of response, time-lapse motion pictures were made to determine how soon after exposure growth was accelerated on the upper side of the leaf. Motion pictures were taken at the rate of 8 frames per minute and when projected on the screen the speed was approximately 90 times normal. For the purpose of this study, the plants were exposed for 30 seconds to vapors of methyl α-naphthaleneacetate, placed before the camera, and the time-lapse pictures were started immediately. Motion pictures were also made of plants responding to ethylene gas. In this case

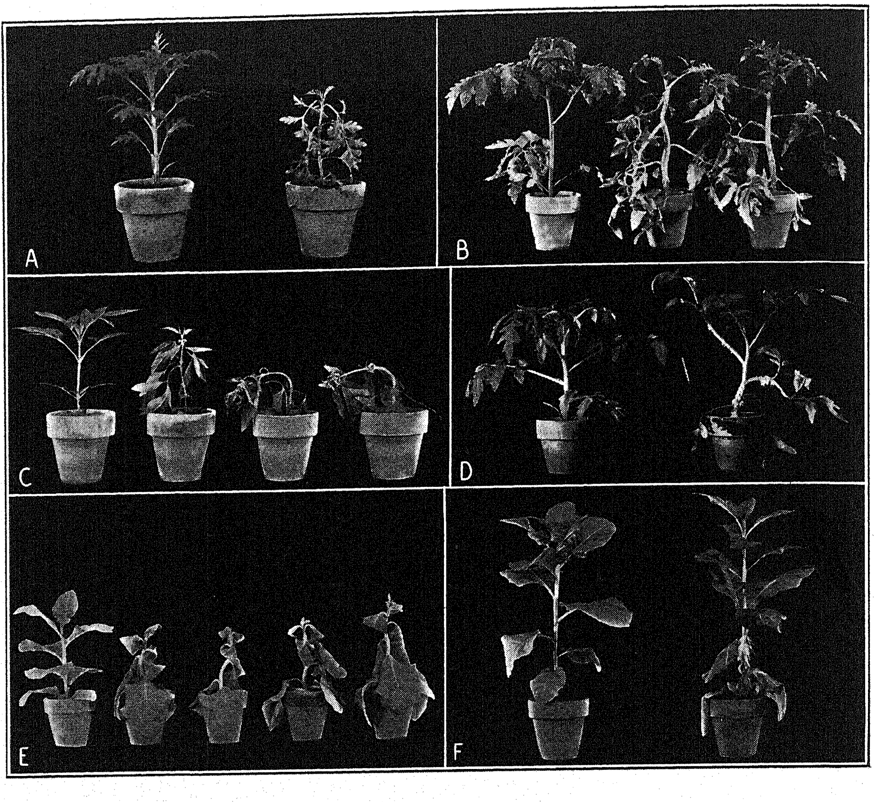

FIGURE 1. Responses of plants to vapors of growth substances. A. Cosmos. Left, control. Right, exposed to ethyl phenylacetate for 24 hours. B. Tomato plants. Left, control. Middle, exposed to methyl naphthaleneacetate for one minute. Photographed 7 days later. Right, exposed to methyl indoleacetate for one minute. Photographed 7 days later. Both treated plants showed pronounced epinasty 24 hours after treatment. Note difference in degree of recovery. C. Spanish needle. Left to right: (1) control; (2) exposed to ethyl indoleacetate; (3) exposed to ethyl naphthaleneacetate; (4) exposed to ethyl β-naphthoxyacetate. Photographed after 24 hours. Note hyponasty, curling of the petioles, and bending of the stems. D. Tomato plant. Left, control. Right, exposed to methyl indolebutyrate. Photographed 9 days later. Note partial recovery and rooting on leaves and stem. E. Tobacco plants showing response to several vapors of growth substances. Photographed 72 hours after treatment. Left to right: (1) control; (2) exposed to methyl indoleacetate; (3) exposed to methyl indolebutyrate; (4) exposed to methyl naphthaleneacetate; (5) exposed to a low concentration of methyl naphthaleneacetate. Note epinasty, twisting of the petiole, and downward curling of the plant. F. Tobacco plants showing roots induced with vapors of methyl indolebutyrate. Left, control (plant a few days younger than treated plant). Right, photographed 22 days after exposure to vapors. Note recovery and growth of the terminal portion and persistence of leaf curling.

TABLE I

RESPONSES OF PLANTS TO VAPORS OF THE MOST ACTIVE GROWTH SUBSTANCES. THE EXPERIMENTS INVOLVED THE METHYL AND ETHYL ESTERS OF THE FOLLOWING ACIDS: α-NAPHTHALENEACETIC (NA), β-NAPHTHOXYACETIC (NOA), INDOLEACETIC (IA), INDOLEBUTYRIC (IB), PHENYLACETIC (PA). ETHYLENE (ETH) WAS USED IN SOME BUT NOT ALL TESTS

Plant	Substance	Responses
Alfalfa	Eth NA IB IA	Epinasty and stem bending. More pronounced in vapors.
Althea	IA IB	Hardwood cuttings rooted throughout entire length but buds were inhibited. Callus formation accelerated at all cut surfaces.
Ardisia	NA	Curling and twisting of young stems and leaves. Retards development of buds, but does not interfere with development of fruit after pollination.
Bean, Windsor (seedlings)	Eth NA IB IA NOA PA	All induced the well known "triple response." The vapors also induced roots on stems.
Bittersweet	Eth NA IB IA	Epinasty with all. Curling of leaves with NA.
Bladder-nut	NA	First hyponasty of entire leaf, then epinasty and curling of leaflets, persisting for several weeks. Floral parts last longer than check, but no parthenocarpy.
Chrysanthemum	Eth NA IB IA	Epinasty and curling of blades in all but ethylene.
Cissus (tropical grape)	NA NOA IB IA	Retardation in rate of elongation of aerial roots and induction of swelling and adventitious roots.
Corn (seedlings)	NA IB IA PA	Abnormal geotropism, curling and twisting, accelerated growth of coleoptiles, and induced roots on mesocotyls.
Cosmos	Eth NA IB IA PA	Epinasty in all treatments. More curling and twisting of petioles with vapors.
Eggplant	NA	Hyponasty and stem bending followed by recovery and epinasty.
Euonymus	NA IB IA	Epinasty, curling of leaves, and stem bending in all. Cuttings rooted in IB.

TABLE I—*Continued*

Plant	Substance	Responses
Fuchsia	NA	Epinasty, curling of leaves. Retardation of floral development, induced parthenocarpy.
Golden chain	NA	Curling, twisting, and hyponasty followed by epinasty. The floral parts (peduncle and pedicels) remained in good condition 35 days. No parthenocarpy.
Heliotrope	Eth NA	NA caused pronounced epinasty and curling of leaves, while Eth did not.
Holly, American	NA IB IA PA	Parthenocarpy and prolonged life of floral parts with NA. Others not especially effective.
Honeysuckle	NA	Epinasty with MNA and fruit set of pollinated flowers.
Hydrangea	Eth NA IB IA	Epinasty of second pair of leaves in all cases. Stem bending and curling in MNA. Retardation of flower bud development in NA and leaves became darker green than controls.
Lilac	Eth NA IA	Epinasty in Eth and hyponasty and stem bending in NA and MIA.
Orchid	NA IB	Pedicels moved downward after treatment as pollinated flowers. The seed pod showed signs of parthenocarpy where treated with NA.
Pea (seedlings)	Eth NA IB IA NOA PA	All induced the well known "triple response." The vapors also induced roots on stems.
Pepper	NA IB IA	Epinasty, twisting in NA. Buds turned yellow and dropped.
Petunia	NA IA IB	Bending of peduncles in all. Epinasty of leaves in NA
Phlox	NA IA IB	Curling of flowers and leaves in NA. No effect on leaves from IA and IB. Flowers opened abnormally small.
Privet (cuttings)	NA IB IA	NA induced roots on cuttings.
Rose, tea and Paul's Scarlet	NA IB IA	Not much effect from IB and IA. Epinasty and browning of leaves and yellowing of buds in NA.

TABLE I—*Continued*

Plant	Substance	Responses
Sensitive plant	Eth NA IB IA	All compounds induced plants to become sluggish or desensitized, ethylene being most effective. Leaves appeared ruffled and assumed an abnormal equilibrium position.
Spanish needle	NA NOA IA IB Eth	Unusual curling and twisting of leaves. Stem bending in vapors.
Strawberry	NA IB IA	Epinasty of leaves. Parthenocarpic development of receptacle without achenes when treated with NA.
Strawberry geranium	NA	Downward curling of leaf blades.
Tobacco	Eth NA IB IA	Hyponasty of young leaves. Epinasty and curling of other leaves when vapors were used, but not with Eth. Vapors induced roots on stems and leaves.
Tomato	Eth NA IB IA NOA	Epinasty of all leaves. Roots induced on stems and leaves. Positive geotropism of horizontal stems in all but Eth.

the plants were enclosed with a trace of ethylene in a glass chamber while being photographed. When projected on the screen, the pictures showed that tomato plants started responding within 20 minutes and tobacco plants 30 minutes after being exposed to the vapors. About 40 minutes elapsed before tomato plants responded to ethylene gas. All except the oldest leaf of the tomato plants (approximately six inches in height) responded at the same time and continued for six hours until the lowermost leaves pressed against the pot. The three youngest leaves curled downward throughout their entire length. The oldest leaves remained nearly straight except at the base where they made a sharp angle with the stem. The second noticeable response was bending of the stem. Within an hour after exposure the stem bent at a 45° angle toward the camera. It was previously noted (6) that when soil of potted plants was treated with water solution of growth substances the plants usually bent toward the light source. The same thing occurred when the plants were exposed to growth substance vapors unless the dosage was too great.

Tobacco plant responses differed from the tomatoes in that the two or three youngest leaves at first moved upward (hyponasty) while the others showed epinasty from exposure to the vapors. The young leaves also moved downward about three hours after exposure to the vapors and all leaf blades curled down at the edges. Though slower at first to respond than the tomato plants, the epinastic movement and curling of leaves continued until the leaves became wrapped around the stem (Fig. 1 E).

The tobacco plants used in these experiments did not show an epinastic response when exposed to ethylene gas while tomato plants in the same chamber showed the usual pronounced epinasty. Some varieties of tobacco respond to ethylene gas and to vapors of other growth substances.

The striking difference between the responses of plants treated with vapors of growth substances or ethylene gas in contrast to local applicaton is the uniform or systemic effect of the former. The nearest approach to the response to vapor occurred when the soil of potted plants was moistened with water containing growth substances. In this case the effects were first local but finally became systemic.

When one leaf of the tomato plant was covered with a flask containing ethylene gas (approximately 1 to 100,000) and photographed at intervals, the resulting time-lapse motion pictures showed the entire plant affected about the same time, though the youngest leaves moved first. A similar experiment involving vapors or a water solution (1 to 100,000) of methyl naphthaleneacetate instead of ethylene showed local effects, where the treated leaf was attached to the stem, followed by systemic effects. These results seem to indicate that ethylene gas moves through the plant more readily than vapors of growth substances when applied locally.

Emanations from treated plants. In the first attempts to make motion pictures of a tomato plant exposed to vapors of growth substances the control plant also showed a pronounced epinastic response. Since the experimental plant had been treated outside the studio it was unlikely that direct vapors from the exposure chamber had come in contact with the control plant. It appeared that emanations from the treated plant must have affected the control. To test this hypothesis various test objects were placed under bell jars with plants which had been exposed previously to vapors of several growth substances. In all cases the test objects quickly showed epinasty of leaves, the final response being nearly as pronounced as that of the treated plants. Later experiments showed that it was not necessary to confine the plants under bell jars. When standing on the laboratory table within a foot or more of treated plants the test objects responded quickly. Four potted tomato plants approximately eight inches in height were exposed to vapors of methyl indoleacetate and then placed with four similar control plants in a glass case of 16 cu. ft. volume. The control plants showed the epinastic response within six hours and roots showed on the stems and leaves of both lots within eight days (Fig. 2). Roots were induced on leaves of treated plants more abundantly than on the controls. In another case where the plants were confined under bell jars with heavily-treated plants the leaves of controls also produced many roots. Attempts are being made to identify the emanations chemically. There is some qualitative evidence that the emanations contain at least some of the same substance applied to the experimental plants. The size and color of roots induced with naphthalene and indole growth substances differ. Those induced with naphthalene compounds are large of diameter and have a pinkish tinge. The roots induced with indole growth substances are white and of small diameter. These same differences persisted where roots appeared on test objects stored with plants treated with vapors of the respective growth substances.

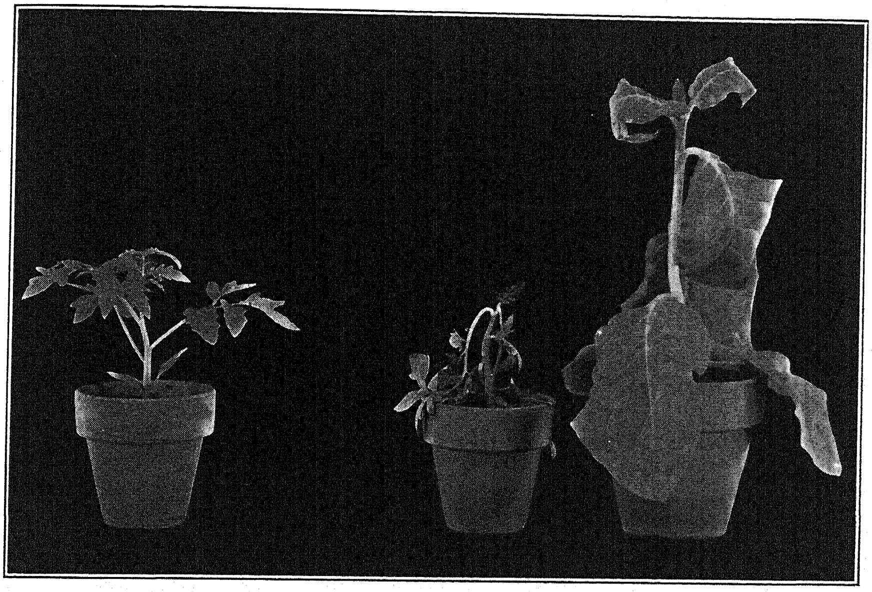

FIGURE 2. The production of emanations by a tobacco plant exposed to vapors of methyl naphthaleneacetate. After airing the treated tobacco plant for one hour, it was placed under a bell jar with a test object (tomato plant), but the two plants were not in direct contact with one another. The test object responded within a few hours and was photographed after 24 hours. The control test object is on the extreme left.

Guttation. When plants are brought from the greenhouse to the laboratory and placed under bell jars, droplets of water soon accumulate along the edge of the leaves. This response, known as guttation, is thought to be due to root pressure after the transpiration rate is reduced.

The amount of water exuded from the leaves when the plants were treated with vapors of growth substances varied with the chemicals and the concentration. Higher dosages of naphthalene and indole substances decreased guttation. Low concentrations though high enough to induce pronounced epinasty did not appear to reduce guttation and were thought at times to increase it. This is in contrast to the effect of applying the same substances in solution to the soil. Ten to fifty mg. of indolebutyric, indoleacetic, or naphthaleneacetic acids in 50 cc. of water applied to the soil of a four-inch pot completely stopped guttation of six-inch tomato plants. Plants treated with vapors and solutions of the same chemicals placed together under one bell jar all showed pronounced epinasty. In no case did those having soil treatment show guttation.

Influence on metabolism. Change in the rate of respiration of treated plants has been studied by absorbing and measuring the carbon dioxide produced. The plants were exposed to vapors of growth substances for five minutes after which the soil was washed from the roots. The plants with roots in water were then placed in a dark, closed system so that air could be drawn through soda lime over the plants and bubbled through barium hydroxide. The hydroxide was titrated at the end of each run and the carbon dioxide produced calculated on the basis of green weight per kilogram per hour. The measurements were made at four time periods called runs. Each period was one and one-half to two hours in length. Eight different tests were made. Two of these tests are shown in detail in Table II. The results of four tests are shown as averages in Table III. The time intervals for the other tests were of different lengths and are not included. However, the first runs showed a lower rate of respiration for the treated plants, but succeeding runs showed that the treated plants respired more than controls.

Roots induced with vapors. Vapors of growth substances induced roots on stems and leaves of intact plants, on hard and softwood cuttings, and on aerial roots of tropical grape. Figure 1 B, D, and F shows several examples of roots induced on intact plants. The same qualitative differences in kinds of roots induced and comparative effectiveness of the substances applied in solution were also evident from exposure to the vapors of the same compounds. The tomato roots induced with vapors of indolebutyric acid or its esters usually resembled normal roots in size and color, whereas tomato roots induced with the naphthalene compounds were of larger diameter and when growing in relatively high humidity were of a pinkish cast. Where color was not involved as with *Kalanchoe* plants the difference in size still held.

Of the entire list of chemicals tested the most active were α-naphthaleneacetic, β-naphthoxyacetic, β-indoleacetic, β-indolebutyric acids and the esters of these acids. Of these four groups the first two and last were the most active for inducing roots on stems and leaf blades of tomato and Turkish tobacco plants. Bausor (1) first reported β-naphthoxyacetic acid as an active growth substance when applied in water and lanolin. Under the most favorable conditions roots appeared from midribs and secondary veins on both sides of the leaf blades. Attached tomato petioles produced roots readily when exposed to vapors of all four groups of substances. The same was true of stems of practically all actively growing plants exposed to vapors. The hydrangea was an exception, responding only to the naph-

TABLE II

RATE OF METABOLISM OF TOMATO PLANTS AS INDICATED BY CARBON DIOXIDE PRODUCTION. PLANTS WERE EXPOSED TO VAPORS OF NAPHTHALENEACETIC ACID FOR FIVE MINUTES AFTER WHICH THE SOIL WAS WASHED FROM THE ROOTS AND THE PLANTS SET IN BEAKERS WITH WATER. THEY WERE PLACED UNDER DARKENED BELL JARS TO MAKE A CLOSED, LIGHT-PROOF SYSTEM THROUGH WHICH AIR COULD BE DRAWN

Run No.	Time		Time elapsed during runs in minutes	Amount of CO_2 absorbed per kilogram of tissue per hour, mg.		Average, mg.	
	Start of run	End of run		Control (in dup.)	Treated (in dup.)	Control	Treated
Test A—Average weight of plants per lot, 115 grams							
1	10:15	11:45	90	232 183	182 182	208	182
2	11:45	1:30	105	243 252	268 319	248	294
3	1:30	3:15	105	265 321	332 310	293	321
4	3:15	4:45	90	287 248	319 333	268	326
Test B—Average weight of plants per lot, 73 grams							
1	10:15	11:45	90	234 232	170 161	233	166
2	11:45	1:30	105	265 303	369 328	284	349
3	1:30	3:15	105	235 299	324 268	267	296
4	3:15	4:45	90	247 340	278 330	293	304

TABLE III

SUMMARY OF FOUR RESPIRATORY TESTS SHOWING THE AVERAGE CO_2 MEASURED AT FOUR TIME PERIODS AFTER EXPERIMENT STARTED

Run No.	Average amount of CO_2 absorbed per kilogram of tissue per hour, mg.	
	Control	Treated (NA vapors)
1	251	197
2	253	370
3	259	322
4	275	319

thalene compounds. It should be stated, however, that the indole compounds were tried in only two concentrations.

Hydrangea and tobacco stems were reported to grow roots at the region of elongation when exposed to carbon monoxide (11), acetylene and ethylene (12). The vapors of the naphthalene substances induced roots from tip to base of growing shoots of these two types. This shows at least qualitative differences between growth substances.

To the "triple response" of etiolated peas and beans can now be added induced rooting when exposed to vapors of growth substances. Most roots arise from the swollen portion of pea stems, but the Windsor bean stem produces roots from tip to base after exposure to vapors of the most active substances. Etiolated corn seedlings root profusely from the mesocotyl. There usually appeared two rows of roots on opposite sides of the central vascular system of the mesocotyl. The tissue between the vascular system and the epidermis became fluffy and the cells separated easily from one another. This was similar to the cortex of Windsor bean and pea stems a few days after exposure to the vapors. When placed in water the cells separated and floated as individuals.

Cuttings of privet and *Euonymus* were exposed for 30 minutes under a bell jar to vapors of the methyl esters of indoleacetic, indolebutyric, and naphthaleneacetic acids. Since these species are commonly used as test objects for determining the relative effectiveness of root-inducing substances, this experiment served two purposes, first to determine whether vapors could be used to induce roots on cuttings, and second to see if the same qualitative differences occurred as previously reported for the species treated with substances in solution. Figure 3 A shows the results with two species and vapors of two substances. It will be noted that *Euonymus* responded readily to methyl indolebutyrate and privet responded to methyl naphthaleneacetate. In neither case did the cuttings treated with methyl indoleacetate form roots ahead of the check. During the course of the experiment privet cuttings did not root when treated with methyl indolebutyrate and *Euonymus* cuttings did not root when treated with methyl naphthaleneacetate. These qualitative differences were evident when solutions of the substances were used to treat these same species of cuttings (7). Higher dosages would probably act like solutions to induce roots in both species. Vapors of methyl indoleacetate induced roots throughout the entire length of six-inch hardwood cuttings of *Hibiscus* when exposed for 30 minutes under a bell jar. Though roots were induced, vapors of this substance inhibited shoot development. Callus formation at all cut surfaces was greatly accelerated when cuttings were exposed to the vapors of the active substances.

Aerial roots of *Cissus* do not normally form branch roots in air unless the tip is removed; then two or more new roots form near the cut surface.

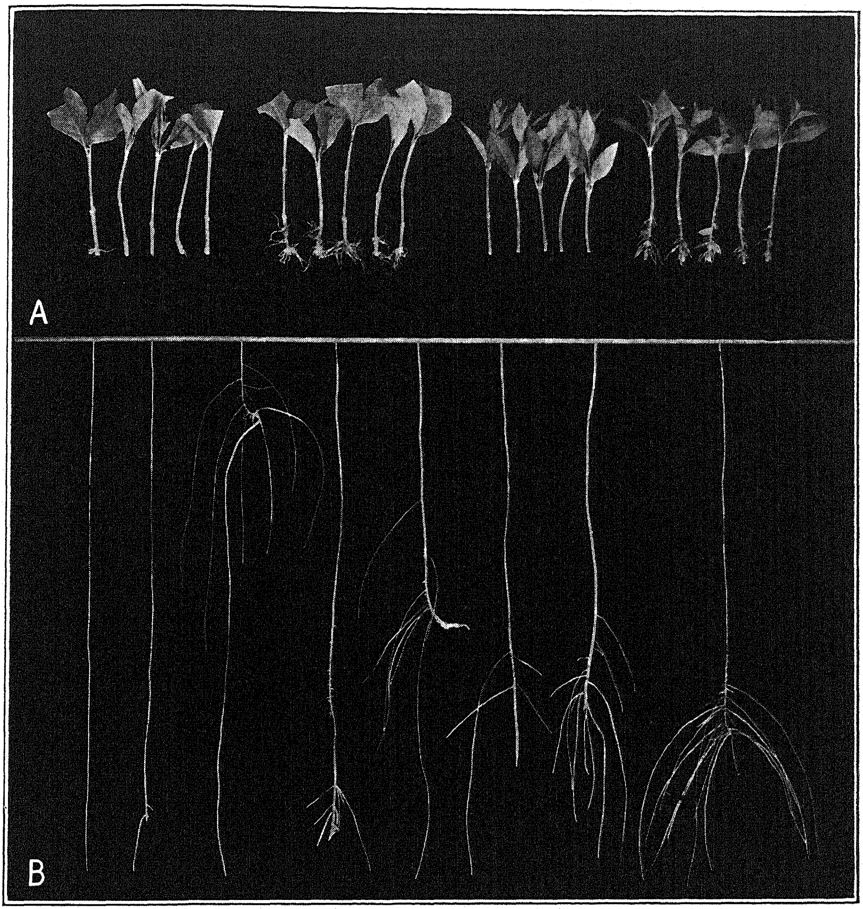

FIGURE 3. Cuttings of *Euonymus*, California privet, and *Cissus* showing roots induced with vapors of growth substances. A. Left to right: (1) control *Euonymus;* (2) *Euonymus* cuttings exposed to methyl indolebutyrate; (3) control privet cuttings; (4) privet cuttings exposed to methyl naphthaleneacetate. B. Aerial roots of *Cissus* exposed to vapors for one minute. Left to right: (1) control showing habit of growth without branch roots; (2) branch roots arising after tip was removed; (3) treated with ethyl naphthaleneacetate when the tip was near the branch roots. Note recovery in growth of original tip. (4) Treated with ethyl indoleacetate when tip was near small branch roots. After the original tip had grown a few inches it was again treated with β-naphthoxyacetic acid. (5) Treated with β-naphthoxyacetic acid. Note induction of branch roots and swelling of the tip. (6) Treated with β-naphthoxyacetic acid in sufficient dosage to kill the tip. Note swelling of the root. (7) Treated with methyl phenylacetate in a high enough dosage to kill the tip; (8) exposed to ethyl indolebutyrate. Photographs were taken 12 days after original treatment.

It was previously reported that if a lanolin preparation of the various growth substances is applied to the tip or region of elongation, many new roots were formed (13). In the present experiments similar results were obtained when aerial roots were exposed to vapors of several substances. The vapors were applied by placing the chemical in the bottom of 16-inch test tubes and then lowering the roots into the test tube so that they extended to a depth of approximately 10 to 12 inches. Usually the roots were inserted through a hole in the rubber stopper which was sealed with modeling clay. When the less volatile substances were used the chemical was warmed with the flame of an alcohol lamp. When heat was applied the roots were exposed for one to five minutes. Vapors of the following substances induced numerous roots when applied in the vapor form: α-napthaleneacetic, β-naphthoxyacetic, phenylacetic, indoleacetic, indolebutyric, *cis* cinnamic acids and the esters of these acids. The results are illustrated in Figure 3 B. These results do not differ greatly from those obtained with growth substances in water solution or lanolin preparations.

The rate of elongation was also retarded as reported for roots treated with solutions of growth substances. Where five roots in each lot were involved they showed the following average elongation in 24 hours: controls, 6 inches; ethyl indolebutyrate, 2 inches; ethyl naphthaleneacetate, 2.5 inches; ethyl indoleacetate, no growth; ethyl phenylacetate, no growth (injury); ethyl naphthaleneacetate, 2 inches. In this experiment roots were initiated from the tip back for a distance of 4 to 6 inches. The primordia could be seen as bumps on the surface within three days after exposure to the vapors. Where tips were killed by the chemicals, many primordia were started back of the dead portion, differing from controls with tips removed which usually produced one or two roots. Considerable swelling accompanied treatments with concentrations high enough to kill the tips. Figure 3 B shows swelling caused by methyl phenylacetate. β-Naphthoxyacetic acid caused pronounced swellings with low concentrations without killing the tip. Bausor (1) reported large swollen areas and root primordia on tomato stems treated with preparations of this substance.

Dormancy and inhibition. It was previously reported that the phenyl growth substances broke the rest period of tubers of *Helianthus tuberosus* while the naphthalene and indole groups inhibited shoot growth from non-dormant tubers (14). Similar results have been reported for potato tubers (5). Figure 4 shows that the rest period of potato tubers was broken with vapors of phenyl compounds. With low concentrations the vapors affected only the terminal bud while higher dosages affected also buds at the base and middle of the tuber. The methyl ester of phenylacetic acid was effective but usually caused considerable injury. This might have been due to over-dosage since the methyl ester is very volatile. Best results were obtained with vapors of phenylacetic acid and the ethyl phenylacetate. *Cis* cinnamic acid also broke the rest period and caused a large number of sprouts to appear. Results are show in Table IV.

Trans cinnamic (phenylacrylic) acid, which is comparatively inactive as a growth substance, was reported to break the rest period of tubers of

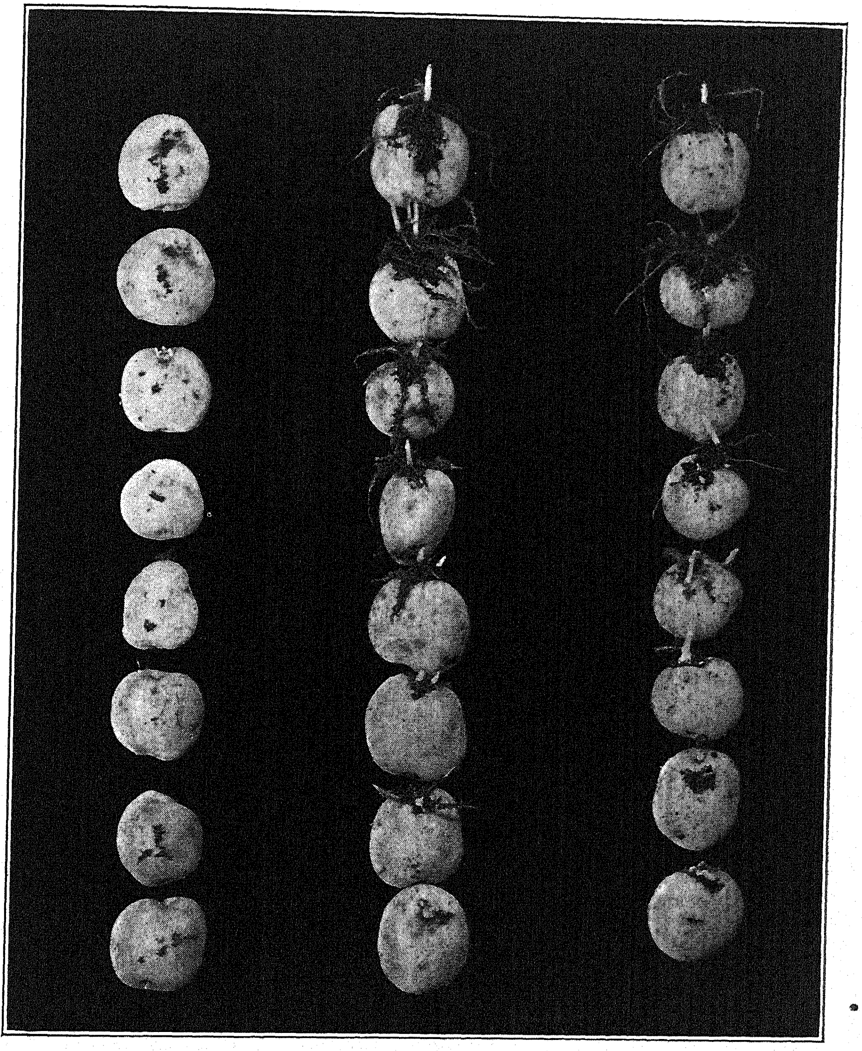

FIGURE 4. Effect of vapors of phenylacetic acid and ethyl phenylacetate on rest period of potatoes grown in Florida and obtained from the market in March. Treated March 31 and planted in peat moss. Photograph taken April 25. Left, control. Middle, exposed for 72 hours to phenylacetic acid. Right, exposed for 48 hours to ethyl phenylacetate. (See Table IV for percentage of sprouting.)

Helianthus (14). The *cis* form (formed when the *trans* form is irradiated) plays a dual rôle; it is an active growth substance according to definition and also breaks the rest period of dormant tubers. Again in contrast with naphthalene and indole growth substances, these have in common with the phenyl compounds the capacity to accelerate growth of tissue through cell elongation, induce epinasty of leaves, and initiate adventitious roots, but the former inhibit development of all buds while the latter break the rest period of dormant buds and have little or no inhibiting effect on non-dormant buds. Since phenyl compounds are natural products of many species of plants, they might prove to be of considerable importance when natural growth regulators are better understood, or when considered from the standpoint of formative effects.

TABLE IV

SPROUTING OF DORMANT POTATO TUBERS* TREATED WITH VAPORS OF GROWTH SUBSTANCES; 8 TO 12 TUBERS IN EACH SET. SPROUTS COUNTED WHEN ONE-FOURTH INCH OR MORE IN LENGTH

Hours treated	Duration of experiment	Substance	% sprouted
48	March 31 to April 25	Control (H_2O vapor)	25
		Phenylacetic acid	100 (See Fig. 4)
		Ethyl phenylacetate	100
50	April 10 to May 17	Control (H_2O vapor)	50
		Cis cinnamic acid	62.5
		Methyl phenylacetate	100
		Ethyl phenylacetate	100
24	April 13 to May 25	Control (H_2O vapor)	12.5
		Naphthaleneacetic acid	0
24	April 24 to May 17	Control (H_2O vapor)	25
		Cis cinnamic acid	37.5
		Phenylacetic acid	87.5
		Ethyl phenylacetate	100
72	April 29 to May 17	Control (H_2O vapor)	33.3
		Ethyl phenylacetate Naphthaleneacetic acid *	83.3
		Ethyl phenylacetate Ethyl indoleacetate **	83.3

* The first three groups listed were white tubers, the other two red.
** Both vapors applied at same time.

In connection with bud inhibition and recovery the differences between naphthalene and indole substances should be mentioned. These differences show clearly in connection with recovery from induced epinasty, resumption of growth of the terminal shoot after exposure to the substances, and degree of bud inhibition. For example, after a given dosage of indolebutyric acid the plant may recover from leaf epinasty in 48 hours, resume growth of the terminal shoot in 96 hours, and axillary buds may be only slightly retarded. An equal dosage of naphthaleneacetic acid or its esters may cause permanent epinasty of leaves, inhibit growth of the terminal shoot for three or more weeks, and prevent the growth of axillary shoots indefinitely. The effectiveness of indoleacetic acid falls somewhere between the two substances used for the illustration. If all of the growth substances were considered, many intergradations would become evident.

Parthenocarpy and retardation of floral development. Inducing seedless fruit to set without pollination has received the attention of a large number of investigators. The peculiar phenomenon occurs commonly in nature and is responsible for such commercial fruits as navel orange, seedless grapefruit, bananas, Chinese persimmon, etc.

According to Gustafson (4), credit is given to Yasuda (9, 10) for having produced the first sizable parthenocarpic fruit experimentally. He used pollen which is known to contain physiologically active substances or extract of pollen as the stimulus. The extracts injected into the ovaries of eggplant or cucumber caused seedless fruit to develop. He also pollinated cucumbers with old or very young pollen to induce seedless fruit.

Gustafson (3) was the first to report the use of well known synthetic growth substances to induce parthenocarpic development. He reported the production of seedless tomatoes, peppers, eggplant, cucumber, etc. Gardner and Marth (2) reported parthenocarpic development of holly berries and strawberries. Wong (8) produced seedless peppers, watermelons, and cucumbers with naphthaleneacetic acid. Several other references to literature on this subject may be found in the publications cited. All of these investigators applied lanolin or other carrier to the stigma or stump of the stigma, or injected material into the ovary. Gardner and Marth sprayed water solutions on the open flowers. Several applications were necessary to obtain a high percentage set. They reported indoleacetic, indolebutyric, and naphthaleneacetic acids effective, but the naphthalene compound was best followed by indolebutyric acid.

The present investigations concern induced parthenocarpy with vapors of growth substances applied to open flowers and flower buds of *Ilex opaca* (American holly), fuchsia, orchid, etc. The potted plants were placed under bell jars and exposed for various lengths of time to vapors of methyl and ethyl esters of naphthaleneacetic, indolebutyric, indoleacetic, and phenylacetic acids. The entire plant was thus exposed. In a few cases only the flower shoot was exposed in a large test tube.

Holly plants used in the experiments were grown from cuttings started in 1937 and 1938. Potted holly flowers freely, and being unisexual was particularly fitting for these experiments. The staminate and pistillate types were kept in separate greenhouses in order to prevent insect pollination. At the time the plants were selected the flower buds were well developed, but only a few had open flowers. The flowers of American holly do not open all at one time. The earliest flower may be a week or more ahead of other flowers on the same branch. This condition was fitting for this experiment since it permitted of treating the buds in different stages of development.

The plants were divided into three lots so that some could be retreated at intervals. At the beginning all except the controls were treated with the esters of the substances listed above. One week later part of the plants were retreated. This lot was divided and a third treatment given half the plants one week later.

The control plants went through the usual flowering period and being without pollination, the floral parts dried up and dropped in two to three weeks.

The plants exposed to the indole substances appeared to hold their flowers slightly better than the controls. There was some enlargement of the ovary from the first treatment, and the lots receiving retreatments were slightly larger than those having only one exposure. However, at the end of 45 days, all floral parts had fallen except from one plant treated with methyl indolebutyrate. This plant showed some development of the ovaries, but there was no permanent set.

Of the plants treated with the phenyl esters, one plant showed definite parthenocarpic development from two flowers, all others having fallen. At the end of the 42-day period only one berry remained and it was smaller than those where natural pollination occurred.

The most striking results were obtained with the esters of naphthaleneacetic acid. One hundred per cent of the treated flowers set fruit. The difference between the controls and treated plants in the early stages is illustrated in Figure 5. The results were the same regardless of whether the plants were exposed to the vapors when the flowers were open or in the bud stage. The fruit set in all cases, but development was slowest when buds were very small at the time the vapors were applied.

As shown in Figure 5 parthenocarpic development occurred without opening of the flowers. The petals continued to clasp the berries and maintained their normal color for 45 days. The petals of a pollinated flower withered within a few days. Very young buds when treated remained at a standstill for two to three weeks, but finally caught up to the others in size of ovaries.

In some cases the holly leaves were damaged by the vapors of methyl naphthaleneacetate. The new leaves on the flower shoot were comparatively resistant, while the year-old leaves turned brown two to three weeks after exposure to high dosages. These damaged leaves did not fall as is characteristic for holly exposed to ethylene gas or smoke. The growth substance apparently interfered with the formation of an abscission layer.

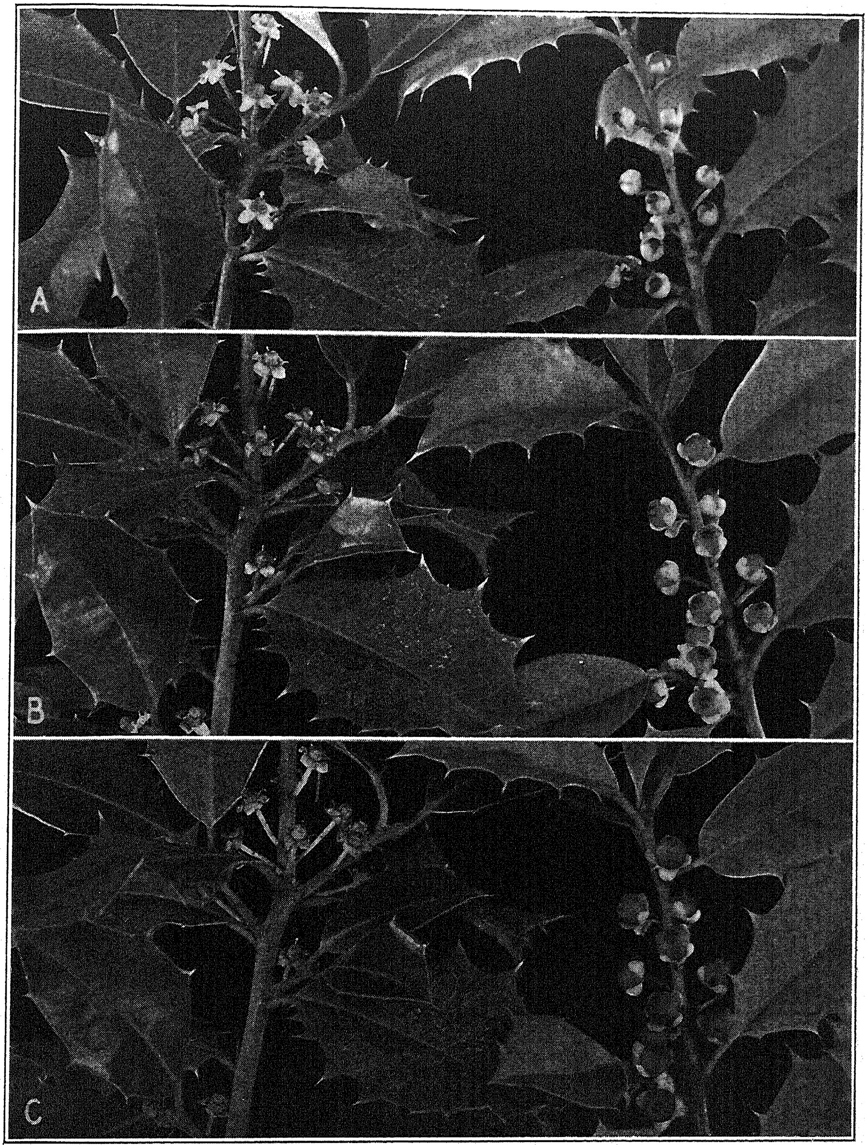

FIGURE 5. Holly, showing parthenocarpic development after exposure to vapors of methyl naphthaleneacetate. A. Left, control. Right, plant exposed on May 17 and photographed on May 26. B. Same shoots as (A) photographed on June 2. C. Same shoots as (A) photographed on June 8. Note withering of floral parts of controls and parthenocarpy and persistence of petals of treated flowers.

The tops of four holly trees (4 to 5 feet in height) were exposed to methyl naphthaleneacetate under a large bell jar when flower buds were beginning to open. The plants were then covered with cheesecloth to prevent natural pollination. The results were essentially the same as described for potted plants.

In one experiment holly plants were selected when approximately half of their flower buds were open and after hand pollination they were exposed to vapors of methyl indolebutyrate, methyl indoleacetate, and methyl naphthaleneacetate. Fruit set occurred in all cases where the open flowers were pollinated regardless of the treatment. The presence of growth substances apparently does not interfere with fertilization. However, the flowers in the bud stage, when treated, set fruit only where the plants were exposed to the esters of naphthaleneacetic acid. It is difficult to explain the failure of vapors of the indole compounds to induce parthenocarpy of holly. These substances were shown by Gardner and Marth (2) to be effective when applied in solution to open holly flowers. These authors, however, reported naphthaleneacetic acid to be the most effective substance.

The present report shows outstanding qualitative differences between the most effective growth substances. Only the naphthalene substances have the capacity to induce parthenocarpy when applied in the vapor form. Also the naphthalene group of substances causes petals to remain intact and keep their color for 45 days. Small buds were definitely retarded though eventually they set fruit without opening the petals. The developing ovaries frequently lifted the clinging petals away from the receptacle.

Fuchsia plants with open flowers and buds were exposed to vapors of ethyl naphthaleneacetate for 30 minutes, and then removed to the greenhouse with the controls. The control plants dropped their flowers, abscissing at the base of the peduncle. The treated plants lost only the corolla and developed a fleshy fruit. The effects of the vapors are illustrated in Figure 6 B. Development of flower buds was retarded. In a few cases the ovaries became fleshy, though the petals did not open. The very youngest buds were killed with dosages which induced parthenocarpy of older flowers.

The orchid (*Epidendrum* O'Brienianum) flowers seldom set fruit when grown in the greenhouse. Over a period of five years only two seed pods have been observed. As soon as pollination is effected, the flower turns downward and the seed pod begins to enlarge. The flowers can be artificially induced to grow downward and develop seed pods by exposure to vapors of ethyl naphthaleneacetate. This response is illustrated in Figure 6 A. Some of the buds failed to open after treatment, but they grew downward shortly after treatment the same as pollinated flowers. Esters of indolebutyric, indoleacetic, and naphthoxyacetic acids also induce the

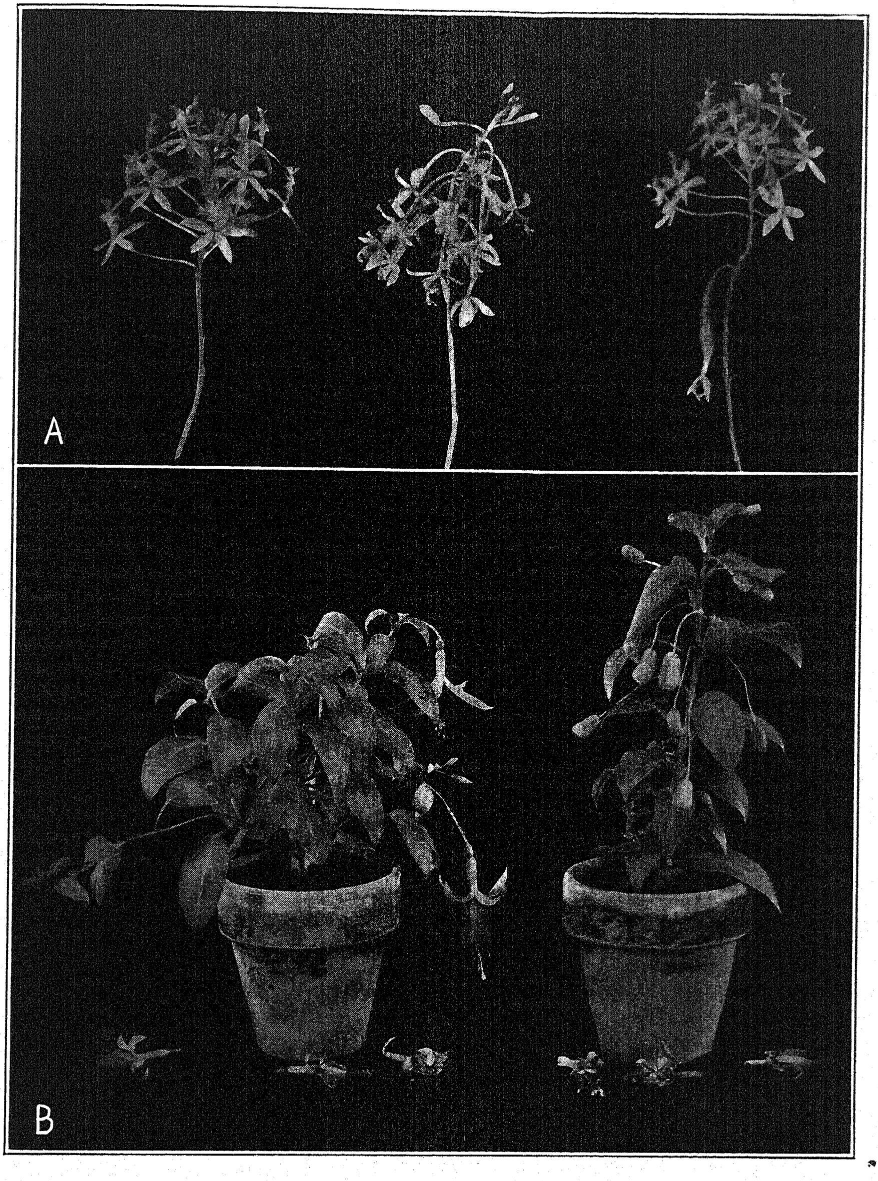

FIGURE 6. Effect of vapors of ethyl naphthaleneacetate on flowers of hybrid orchid and fuchsia. A. Orchid. Left, control. Middle, exposed to vapors for one minute and photographed after 5 days. Note downward growth of flowers. Right, illustration of the downward growth of seed pod after natural pollination. B. Fuchsia. Left, control showing normal habit and abscission of flowers. Right, plant exposed to vapors for 10 minutes. Photographed 27 days later to show parthenocarpic development of ovaries. Corollas abscissed at the base of the tube, leaving the enlarged ovaries attached to the plant.

flowers to turn downward after exposure to vapors. At this date they have not had time enough to develop seed pods.

By treatment with vapors of methyl naphthaleneacetate strawberry flowers which had not been pollinated were induced to develop receptacles which ripened in the normal way, but had few or no achenes. The shape of the ripened receptacles was more slender than normal fruit with seeds.

Flower buds of tomatoes exposed to vapors of ethyl naphthaleneacetate set fruit without opening but to date no sizable fruit has been obtained. It is difficult to treat flower buds of tomato with vapors without at the same time greatly affecting the plant as a whole.

Many other plants with flower buds have been treated with vapors of growth substances but no other striking results have been obtained. In general it might be said that the naphthalene substances retard the development of flower buds and induce parthenocarpy in the species which normally have fleshy fruit.

Effect on seedlings. It was recently reported that seedlings of pea and corn made an abnormal geotropic growth when treated with vapors of growth substances (16). In the present tests Windsor beans, garden peas, oats, and corn seedlings were exposed to vapors of naphthalene, naphthoxy, indole, and phenyl substances. The naphthalene acids and esters were the most active substances followed by indoleacetic and then indolebutyric acids and esters.

Windsor bean responses resembled those of the pea except that swelling was more uniform along the stem instead of being localized at the tip (Fig. 7 B). The rudimentary leaves of the etiolated beans elongated, became curled and twisted, assuming the shape of a corkscrew. In some cases these rudimentary leaves produced tendrils which also coiled. The cortex of the stems became spongy and the cells separated from one another and from the central cylinder. Roots were produced in large numbers along the stem of the treated plants. Young roots growing above ground and in the ground formed knobs immediately back of the growing point when the seedlings were treated with vapors of the naphthalene substances. In many cases bending occurred especially at the first node, but curling also occurred frequently between the nodes.

The vapors of all active growth substances including unsaturated carbon gases applied to pea seedlings caused the triple response—abnormal geotropism, retardation in rate of elongation, and swelling near the stem tip (16). The degree of the responses varied with the substances as with Windsor bean and corn seedlings. Sections through the swollen region showed that the cells were much shorter than those of controls, but two or more times the normal diameter.

Seedlings of corn and oats exposed to the vapors curled and twisted along the mesocotyls and coleoptiles. The coleoptiles of oats sometimes

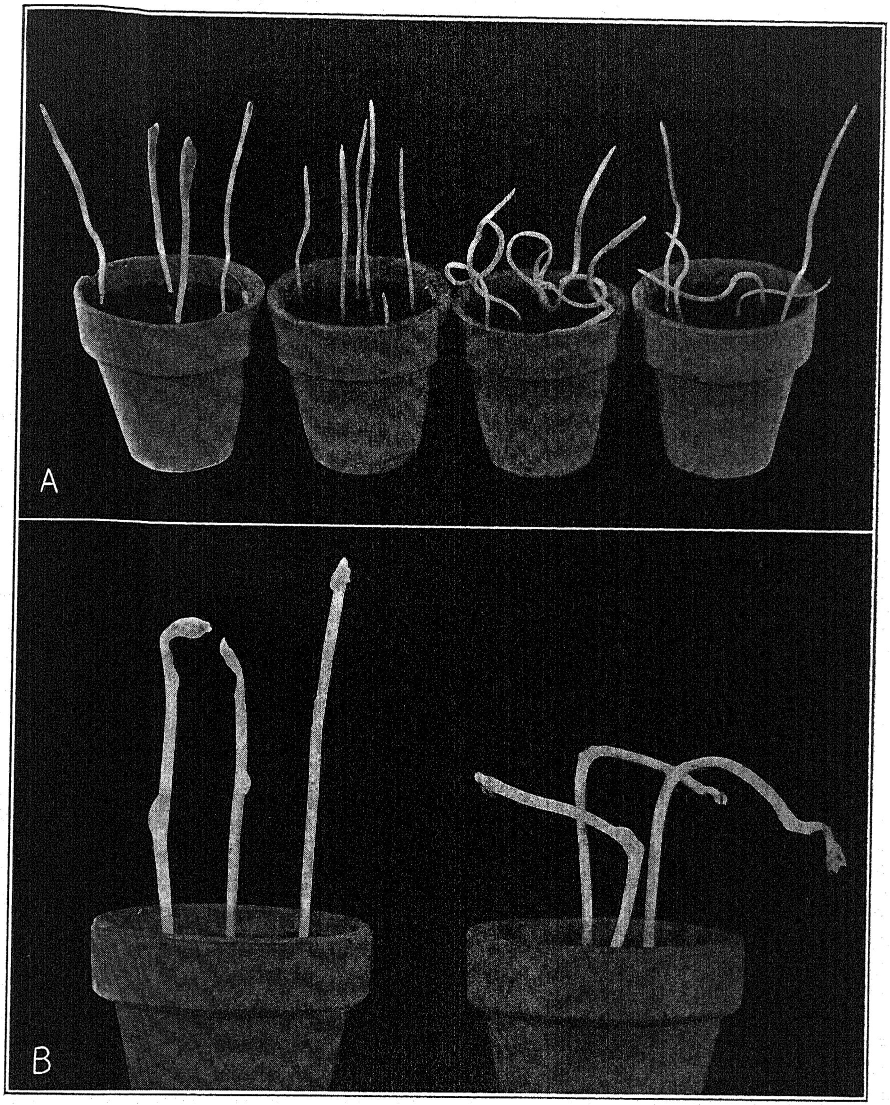

FIGURE 7. Effect of vapors of growth substances on seedlings of corn and Windsor bean. A. Corn. Left to right: (1) control in light 24 hours; (2) control remaining in the dark; (3) seedlings exposed to methyl naphthaleneacetate and then placed in light for 24 hours; (4) seedlings exposed to methyl indoleacetate and placed in light for 24 hours. Note the control in light shows first leaf broken through the coleoptile, while treated seedlings in light still have intact coleoptiles. B. Windsor bean. Left, control. Right, plants exposed for 10 minutes to methyl naphthaleneacetate and photographed after 20 hours.

coiled like a corkscrew. There appeared to be no regularity as to where the curling occurred and occasionally a seedling remained straight and upright after exposure to the vapors (Fig. 7 A). Bending frequently occurred at the nodes. These usually enlarged during the first 48 hours after treatment followed by the production of roots. The mesocotyl of corn showed occasional swollen regions. The entire mesocotyl became enlarged, and the cortical layer became spongy as described for Windsor bean. Roots were induced in two rows along the central vascular strand.

Coleoptiles of oats and corn showed abnormal elongation when exposed to the vapors in light or dark. The first leaf broke through the coleoptiles of control seedlings shortly after being exposed to light. The coleoptiles of treated plants grew faster than the leaf in both light and dark. The leaves often remained inside the coleoptile for 48 hours or more after exposure in light. When the coleoptiles curled, the leaves frequently broke out at the base instead of the top of the coleoptiles. There was considerable variation in the length of coleoptiles among controls of the same age. This variation was apparent also after the seedlings were exposed to vapors of growth substances.

DISCUSSION

Plants treated with vapors of a given substance respond quicker than those treated with solutions of the same compound. This brings up the question of penetration. There is a possibility that substances applied as vapors enter the tissue in the gaseous state the same as ethylene gas.

Several compounds which caused epinasty when applied in vapor form, caused no effect or positive bending when applied in lanolin. The following are examples: naphthylamine, naphthoflavone, phenylethylacetic acid, phenylglycine ethyl ester, pimelic acid, nicotinic acid nitrate, ethyl bromoacetoacetate, methyl ethyl phenylethylmalonate.

Some compounds which were on the border line for activity or inactivity caused curling along the entire leaf instead of pronounced bending at the base of the petiole which is typical for ethylene gas.

Under the heading "Material and Methods" phenylbutyric acid is listed as a new substance, since it has never been previously used as a vapor or reported as inducing epinasty although often mentioned in connection with growth substances. Under the conditions of our experiments the vapors of phenylbutyric acid caused epinasty of tomato leaves in the course of three hours.

A new chemical prepared in the research department of Merck called methyl trimethylamino indole propionate iodide deserves special mention. This substance was not active when applied locally as a lanolin preparation regardless of concentration. When heated under a bell jar with test objects, vapors arose and the plants responded quickly indicating physiological activity for the substance. Since, however, this compound is not active in solution, it might be assumed that its composition was changed, perhaps losing the methyl iodide and resulting in an active substance, methyl dimethylamino indole propionate. The manner in which the plants responded to vapors of this substance was striking. The lowermost leaves twisted and turned upside down, while the upper leaves moved downward, characteristic of induced epinasty with other substances. Enough of the active chemical clung to the wall of the bell jar to induce a pronounced response of test objects for three weeks after the first exposure. The responses were always characteristic and different from that of other substances. An attempt is being made to determine the exact structure of the active substance.

The picrate salts of the growth substances were found active in solution. They are thought not to be stable but under the conditions of our experiments it was impossible to find any differences in the response of these substances applied in solution or as vapors.

The fact that plants fail to show guttation after water solutions of the substances are applied to the soil raises a number of interesting questions. If guttation is associated with root pressure, it would appear that the substances change the capacity of roots to function. The fact that vapors applied to potted plants did not prevent guttation suggests that the substances did not reach the roots quickly.

To treat cuttings they were turned upside down in beakers and placed under bell jars for 30 minutes with vapors. The basal parts formed roots just as if they had been treated with solutions. The same qualitative differences in the substances brought out by applying the compounds in solution became evident when vapors were used. Types of roots and susceptibility of species were clues used to make comparisons.

A point of considerable interest is the fact that flowers or floral parts of holly lasted an abnormally long time after they had been treated with naphthalene growth substances. The petals remained in good condition and kept their color 45 days after buds had been exposed to the vapors. Not all species responded in this manner. Phlox flowers lasted only slightly longer than normal and small buds failed to open after exposure to esters of indole and naphthalene substances. Flower buds that were medium size at the time of treatment opened, but were smaller than normal.

Fuchsia flowers treated with naphthaleneacetic acid vapors lasted five to ten days longer than controls. Control flowers abscissed at the base of the peduncle whereas only the corollas dropped from treated plants, and the ovaries ripened fleshy. This shows an interesting control mechanism associated with fruit set. It seems logical to assume that the regulator is of the nature of a chemical substance. Abscission of the corolla and associated fruit set after natural pollination are the same as that of chemically induced parthenocarpy.

SUMMARY

The experimental results reported in this paper involve 54 physiologically active substances that are designated as growth substances. Of this list of compounds 28 were reported active when applied as vapors in a preceding publication (16), and 26 are now being reported for the first time. The new compounds are as follows: β-naphthoxyacetamid, ethyl β-naphthoxy α-butyrate, β-naphthoxy α-butyric acid, ethyl α-naphthoxy α-butyrate, methyl and ethyl β-naphthoxyacetate, methyl and ethyl α-naphthoxyacetate, α-naphthoflavone, α-naphthylamine, α-naphthaleneacetic acid picrate, β-naphthoxyacetic acid picrate, α-naphthoxyacetic acid picrate, iso-butyl phenylacetate, butyl phenylacetate, methyl ethyl phenylethylmalonate, phenylbutyric acid, phenylethylacetic acid, *N*-phenylglycine ethyl ester, irradiated methyl *m*-nitrocinnamate, methyl α-trimethylamino-β-(3-indole) propionate iodide, ethyl α-bromoacetoacetate, homopiperonilic acid, nicotinic acid nitrate, pimelic acid, and *m*-tolyl β-naphthoxyacetate.

The responses induced with growth substances concerned in this paper are of a formative nature usually affecting particular organs or parts of a plant, differing in this respect from fertilizers. Downward growth of leaves, enlarged stem tips, positive geotropism of stems, hypertrophies, parthenocarpy, and induced adventitious roots are examples of formative effects, which are referred to as induced abnormalities.

There are many qualitative differences in responses induced by the 54 substances, but they have in common the capacity to induce epinasty of tomato leaves. All chemical compounds which have the capacity to induce epinasty of tomato leaves similar to that induced by ethylene gas have been called "growth substances."

Through the use of motion pictures, it was shown that after exposure to vapors of methyl α-naphthaleneacetate, tomato plants responded in 20 minutes, and tobacco plants in 30 minutes.

Plants treated with vapors of the more active growth substances gave off emanations which affected other plants standing near. When treated and then removed to a clean bell jar with a test object, both plants made pronounced responses.

The influence of growth substance vapors on rate of metabolism was studied by measuring the carbon dioxide evolved when plants were in the dark. During the first hour after exposure the treated plants produced less carbon dioxide than controls, but for the next five hours they exceeded the controls.

By exposure to vapors of growth substances many species of plants were induced to form adventitious roots on leaves, stems, roots, and cuttings.

Phenyl growth substances applied in vapor form broke the rest period of dormant potatoes in contrast with the naphthalene substances which inhibited bud growth.

Vapors of methyl and ethyl α-naphthaleneacetate caused parthenocarpic development of holly berries, ovaries of fuchsia, and enlargement of the receptacle of strawberries. Hybrid orchid flowers grew downward when exposed to vapors as if naturally pollinated, and there were indications of seed pod development without pollination. The petals of treated holly flowers and buds remained in good condition for 45 days in contrast to a few days for controls. The ovaries developed without opening of the buds. The naphthalene substances were more effective than indole and phenyl compounds for inducing parthenocarpy.

Vapors of growth substances applied to etiolated pea and Windsor bean seedlings induced abnormal geotropism, retardation in rate of elongation, swelling of stems (especially the tip in peas), and adventitious roots on stems and roots.

Coleoptiles of etiolated oats and corn seedlings showed abnormal elongation when exposed to the vapors under light or dark conditions. The first leaf broke through the coleoptile of controls shortly after being exposed to light. The leaves remained inside the coleoptile of treated plants for 48 hours or more while in light.

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EXPERIMENTS ON CONTROL OF JAPANESE BEETLE LARVAE WITH β, β'-DICHLOROETHYL ETHER[1,2]

Albert Hartzell and Frank Wilcoxon

In a previous publication (6) it was shown that β, β'-dichloroethyl ether could be successfully used in greenhouse fumigation to control a number of insect pests.

The present paper presents the results of experiments designed to test the possibility of using this substance to control Japanese beetle larvae (*Popillia japonica* Newm.) in turf. Preliminary tests made in the spring of 1938 had indicated that dichloroethyl ether offered promise as a soil fumigant against this species. Campbell and Stone (2) in 1937 published a note on the successful use of this substance as a soil fumigant in the control of wireworms (*Limonius* [*Pheletes*] *californicus* Mann). Stone and Elmore (4) described the control of sod webworms, *Crambus* sp., on lawns, by sprinkling infested areas with an aqueous solution of this compound.

MATERIALS AND METHODS

Arrangement of plots. In the fall of 1938 two sets of experimental plots were laid out on a local golf course and two more on a lawn at the Boyce Thompson Arboretum. These will be referred to as experiments A, B, C, and D. In the spring of 1939 a single set of experimental plots (E) was laid out on the golf course. Each experiment consisted of four blocks, each containing four plots. The plots were separated from each other by strips of untreated turf five yards wide. Since there were four treatments applied including a check, there were, in all, four replications of each treatment per experiment. Each plot constituted a square with an area of 25 square yards. The treatments were randomized within the blocks.

Types of treatment. The types of treatment used in experiments A and B consisted of (a) powdered lead arsenate applied at the rate of 10 pounds in 30 pounds of garden soil broadcast over 1000 square feet of turf and raked in to one-fourth inch depth; (b) a saturated solution of dichloroethyl ether in water to which was added 0.1 per cent Tergitol 7 penetrant, a sulphated alcohol (7), applied once; (c) a similar spray as in (b) applied twice about a week apart; (d) check plots which received no treatment.

Experiments C and D were similar to experiments A and B, but the amount of Tergitol 7 penetrant used was increased to 0.5 per cent in C and D.

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[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 195.

[2] The materials for this investigation were furnished by the Carbide and Carbon Chemicals Corporation, New York, N. Y.

Experiment E, which was performed in the spring of 1939, was similar to the other experiments except that a saturated solution of dichloroethyl ether without any wetting agent was compared with a similar solution containing 0.1 per cent Tergitol 7 penetrant and two applications were made in each case.

The saturated solution of dichloroethyl ether (8.36 cc. per l.) was made by adding the necessary amounts of the constituents in a power sprayer (Fig. 1) and running the agitator for 30 minutes. It was necessary to make sure that dichloroethyl ether was in solution, otherwise injury to the grass resulted. The spray was applied at the rate of approximately 25 gallons per plot at a pressure of 150 to 200 pounds. The material may be applied to small areas by means of a sprinkling can. As dichloroethyl ether is a toxic volatile compound, reasonable care must be taken not to inhale the fumes (1, p. 206).

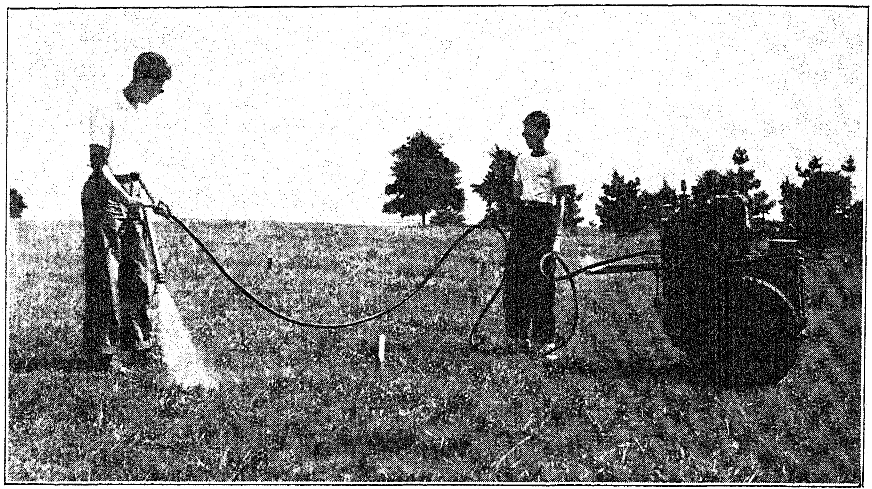

FIGURE. 1. Applying dichloroethyl ether mixture to turf by means of a 50-gallon capacity power sprayer.

The plots were sampled by turning over the sod with a spade until 25 or more grubs had been counted per plot and the number of living and dead larvae were noted. The counts were made about a week after the final application of the spray. Arsenate of lead acts so slowly that no appreciable kill was obtained from it in this short period. Plots treated with lead arsenate in the fall and sampled the following spring (May 1) showed an average decrease in grub population of 72 per cent.

RESULTS

The results of these experiments are shown in Table I. Inspection of this table shows that the dichloroethyl ether solution had a marked toxic effect on the larvae. Furthermore two successive applications gave a significantly higher kill than a single application. Increasing the content of Tergitol 7 penetrant from 0.1 per cent to 0.5 per cent produced a very great increase in the percentage of dead insects but led to injury to the grass. The results obtained in the spring of 1939 indicate that a fairly satisfactory kill may be obtained using 0.1 per cent Tergitol 7 penetrant if two applications are made. It does not seem practicable to use a concentration as

TABLE I

RESULTS OBTAINED IN FALL OF 1938 AND SPRING OF 1939 FROM TREATING TURF WITH AN AQUEOUS SOLUTION OF DICHLOROETHYL ETHER; FOUR PLOTS IN EACH TREATMENT

Treatment of plots	No. of applications	Date applied	Exp. No.	Date counted	No. dead	No. alive	Total grubs counted	% dead	Injury to turf
Lead arsenate	1	Aug. 22	A	Sept. 12	2	107	109	1.8	None
		Aug. 22	B	Sept. 12	5	103	108	4.6	None
		Sept. 22	C	Oct. 6–10	3	101	104	2.9	None
		Sept. 22	D	Oct. 6–10	3	106	109	2.8	None
		April 14	E	May 18	3	101	104	2.9	None
Sat. soln. of dichloroethyl ether+0.1% Tergitol 7 penetrant	1	Aug. 29	A	Sept. 12	7	104	111	6.3	None
		Aug. 29	B	Sept. 12	5	113	118	4.2	None
Sat. soln. of dichloroethyl ether+0.1% Tergitol 7 penetrant	2	Aug. 29*	A	Sept. 12	22	75	97	22.6	None
		Sept. 6*	B	Sept. 12	31	79	110	28.0	None
		May 4 May 11	E	May 18	93	14	107	86.9	None
Sat. soln. of dichloroethyl ether+0.5% Tergitol 7 penetrant	1	Sept. 26	C	Oct. 10	80	37	117	68.4	Slight
		Sept. 28	D	Oct. 6	75	46	121	62.0	Slight
Sat. soln. of dichloroethyl ether+0.5% Tergitol 7 penetrant	2	Sept. 26 Sept. 29	C	Oct. 10	110	4	114	96.4	Severe
		Sept. 28 Oct. 3	D	Oct. 10	90	22	112	80.4	Severe
Sat. soln. of dichloroethyl ether	2	May 4 May 11	E	May 18	30	76	106	28.3	None
Check	0		A	Sept. 12	1	108	109	0.9	None
			B	Sept. 12	0	108	108	0	None
			C	Oct. 10	1	112	113	0.9	None
			D	Oct. 10	0	120	120	0	None
			E	May 18	2	98	100	2.0	None

* Applied too early in season for effective control as not all eggs had hatched.

high as 0.5 per cent but it is possible that a concentration intermediate between 0.1 per cent and 0.5 per cent may give satisfactory control without injury to the grass. Further experiments are needed to settle this point.

SEASONAL OCCURRENCE OF THE INSECT AND CLIMATIC FACTORS

An examination of the grubs in the experimental plots during the spring of 1939 showed that from 78 per cent to 97 per cent of the individuals were the larvae of the Japanese beetle; the remainder were chiefly *Autoserica castanea* Arr. The grubs had begun their migration downward by October 1, 1938, as none was found nearer than two inches below the surface of the soil. On May 1, 1939 they were within one-half inch of the surface. The grub population averaged approximately 25 per square foot of turf in the area treated.

Since the amount of moisture in the soil would have a tendency to dilute the dichloroethyl ether and decrease its toxicity, the amount of rainfall before and after treatment may have a marked effect on its effectiveness. No rain fell during the ten-day period prior to treatment of plots with dichloroethyl ether in experiments A and B (3). At the time the grubs were counted (September 12, 1938) less than one inch of rain had fallen during the course of the experiment. In contrast to this, the late treatments were applied during a rainy period. There was a precipitation of 8.14 inches during the ten days preceding the application of dichloroethyl ether in experiments C and D, while slightly more than an inch of rain had fallen from the time the treatments were applied until October 10, 1938, when the grubs were counted.

Dichloroethyl ether was applied to the plots in experiment E during a relatively dry period in the spring of 1939. Only 0.25 inch of rain fell (5) during the ten-day period prior to the first application, while 0.15 inch had fallen from the time the first treatment was made to May 18, when the grubs were counted. The soil temperature ranged from 10° C. to 24.5° C.

DISCUSSION

It is probable that the lead arsenate treatments would have given a higher kill than reported in the above experiments had the grubs been actively feeding throughout the period. It would appear from these results (Table I) that two treatments of dichloroethyl ether followed two weeks later by a treatment of lead arsenate would combine the advantages of quick action with that of residual effect. The dichloroethyl ether would rapidly kill the grubs already present in the soil, while lead arsenate would tend to prevent reinfestation.

In situations such as public parks and playgrounds where a poisonous residue may be objectionable due to health hazard, dichloroethyl ether may offer an effective substitute for lead arsenate.

SUMMARY

β, β'-dichloroethyl ether has been tested as a soil fumigant against the larvae of the Japanese beetle. The saturated aqueous solution with the addition of 0.1 per cent Tergitol 7 penetrant, a sulphated alcohol, was found to give a satisfactory control of the grubs when two applications were made about one week apart during the period when the grubs were near the surface of the soil. Increasing the amount of Tergitol 7 penetrant increased the effectiveness of the mixture but led to injury of the grass. This mixture offers promise in cases where it is desired to avoid the use of materials which leave poisonous residues, such as lead arsenate, and where a rapid kill is required.

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